On Fermentation by Alonzo Englebert Taylor.

University of California Publications in Pathology. 1907. vol. I, no. 8.

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PATHOLOGY

Vol. 1, No. 8, pp. 67-341 February 16, 1907

ON FERMENTATION

ALONZO ENGLEBERT TAYLOR

BERKELEY

UNIVERSITY PRESS

PRICE $2.00

UNIVERSITY OF CALIFORNIA PUBLICATIONS

Note.—The University of California Publications are offered in exchange for the publications of learned societies and institutions, universities and libraries. Complete lists of all the publications of the University will be sent upon request. For sample copies, lists of publications or other information, address the **Manager of the University Press, Berkeley, California, U. S. A.** All matter sent in exchange should be addressed to **The Exchange Department, University Library, Berkeley, California, U. S. A.**

PATHOLOGY.—Alonzo Englebert Taylor, Editor. Price per volume $2.00

Volume I (in progress).

No. 1. On the Quantitative Separation of the Globulins of Hemolytic Serum, with Special Reference to the Carbon Dioxide Group, by Clarence Quinan. Price, .05

No. 2. Hydrolysis of Protamine with Especial Reference to the Action of Trypsin, by Alonzo Englebert Taylor. Price, .25

No. 3. On the Synthesis of Fat Through the Reversed Action of a Fat-Splitting Enzyme, by Alonzo Englebert Taylor.

No. 4. On the Occurrence of Amido-Acids in Degenerated Tissues, by Alonzo Englebert Taylor. In one cover. .25

No. 5. On the Autolysis of Protein, by Alonzo Englebert Taylor.

No. 6. On the Reversion of Tryptic Digestion, by Alonzo Englebert Taylor. In one cover .25

No. 7. Studies on an Ash-Free Diet, by Alonzo Englebert Taylor. Price, .20

No. 8. On Fermentation, by Alonzo Englebert Taylor. Price, 2.00

The following series in Graeco-Roman Archaeology, Egyptian Archaeology, American Archaeology and Ethnology and Anthropological Memoirs are publications from the Department of Anthropology:

GRAECO-ROMAN ARCHAEOLOGY. (Large Octavo).

Vol. 1. The Tebtunis Papyri, Part 1. Edited by Bernard P. Grenfell, Arthur S. Hunt, and J. Gilbart Smyly. Pages 690, Plates 9, 1902 Price, $16.00

Vol. 2. The Tebtunis Papyri, Part 2 (in press).

Vol. 3. The Tebtunis Papyri, Part 3 (in preparation).

EGYPTIAN ARCHAEOLOGY. (Quarto).

Vol. 1. The Hearst Medical Papyrus. Edited by G. A. Reisner. Hieratic text in 17 facsimile plates in collotype, with introduction and vocabulary: pages 48, 1905. (J. C. Hinrichs, Leipzig, 25 Marks) Price, $8.00

AMERICAN ARCHAEOLOGY AND ETHNOLOGY. (Octavo).

Vol. 2. No. 1. The Exploration of the Potter Creek Cave, by William J. Sinclair. Pages 27, Plates 14, April, 1904 Price, .40

No. 2. The Languages of the Coast of California South of San Francisco, by A. L. Kroeber. Pages 52, June, 1904. Price, .60

No. 3. Types of Indian Culture in California, by A. L. Kroeber. Pages 22, June, 1904. Price, .25

No. 4. Basket Designs of the Indians of Northwestern California, by A. L. Kroeber. Pages 60, Plates 7, January, 1905. Price, .75

UNIVERSITY OF CALIFORNIA PUBLICATIONS

PATHOLOGY

Vol. 1, No. 8, pp. 87-341 February 16, 1907

ON FERMENTATION.

(From the Hearst Laboratory of Pathology)

BY

ALONZO ENGLEBERT TAYLOR.

(Dr. M. Herzstein of San Francisco has established in the University of California a Lectureship devoted to the exposition of scientific subjects fundamental to medicine. The following publication comprises in a condensed form the lectures scheduled for 1904-05, delivered in October, 1904. They include, however, literature references up to December 31, 1905. The publication has been delayed for over a year, an indirect result of the conflagration of April 18, 1906.)

INTRODUCTION.

These lectures represent an attempt at a brief systematic consideration of the question of fermentation from the double point of view of general chemistry and chemical biology. They contain, in addition to a review of the special literature of the topic, unpublished researches on various aspects of the subject. The scope of the discussions is limited to fermentations of importance to the animal economy. And of these fermentations those only shall be considered concerning which we possess data, sufficient in quantity and reliable in quality, that will enable us to rest the discussion upon a rigid objective basis. It must be realized that such an attempt will in reality state the problem rather than define the extent of its present solution, since the data are in truth scant, and many adventitious variables complicate the investigations. It will I believe be advantageous at the outset to state formally the conclusions towards which our theoretical analogies and our experimental researches lead. It may seem anomalous to place conclusions in the introduction. This is justified by the fact that the problem is not one that has arisen directly from the experimental investigations upon fermentations, but is one that has been extended to fermentations as the

result of the modern development of chemical dynamics. The study of fermentation consists in the attempt to verify there the laws that obtain in general chemistry—the attempt to determine in a complex biological material the existence and validity of the laws that have been proved in simple chemical material. Under these circumstances to state the conclusion is equivalent to stating the problem.

Fermentations shall be considered as accelerations of existing reactions. As accelerations of existing reactions they are ranged with the reactions of catalysis. In every reacting system there is a driving force and a passive resistance to the reaction. The catalysor is related only to the condition of passive or internal resistance. Anything that will lower this passive resistance will accelerate the reaction velocity. The positive catalysor and increase in temperature both act by such a reduction in the internal resistance. The modus operandi of catalytic acceleration is in general defined as a succession of intermediary reactions—a definition that applies directly only to reactions in a homogeneous system. Fermentations are considered as limited and reversible reactions. There is no known essential difference between an inorganic positive catalysor and a ferment; there is no distinction in dynamics between so-called formed and unformed ferments. Cells induce fermentations only through the agency of chemical substances elaborated by them. All fermentations tend to obey the laws of chemical kinetics; the experimental deviations are proportional to the multiplicity of adventitious variables and to the difficulty in the definition of the experimental magnitudes. The greater the control in the experiment, the closer the approximation to the theoretical law.

The experimental study of a fermentation may be thus formulated in specific terms:—

The primary reaction. This is a problem of organic chemistry.

Relation of mass of substrate to reaction velocity.

Relation of mass of ferment to reaction velocity.

Relation of concentration in entire system to reaction velocity.

Relation of temperature to reaction velocity. Temperature optimum.

Reversion of reaction by ferment action.

Relation of ferment to the products of reaction.

Auto-acceleration; zymo-excitors; zymo-depressors.

Inactivation of ferment.

Secondary reactions.

Nature of ferment. Colloidal properties. Relation of activity to history and method of preparation.

The control of the conditions—the purity of the reacting components and of the ferment, the temperature, concentration, and the inhibition or inactivation of the ferment—will determine the reliability of the results. It is to be confessed that too often such control has not been attained. The more nearly the experiment approximates the conditions of an ideal chemical experiment, the more credible the results. To be entirely definite the results should be quantitative and capable of a mathematical interpretation. The problem of fermentations differs only in degree from the problem of catalytic reactions, directly in proportion to our inability to study all the aspects as above stated, to control the variables and to define the experimental magnitudes. These conditions have thus far been best attained in connection with the study of the ferments derived from the vegetable or lower orders of animal life.

The statement that fermentations are to be regarded as accelerations of existing reaction demands a certain restriction. The earlier chemists in general regarded catalytic reactions as reactions de novo; the present general currency of the opposite proposition is due largely to the influence of Ostwald and Victor Meyer, though the first theoretical statement was probably contained in the thermodynamic considerations of van't Hoff bearing upon the relations between reaction-velocity and temperature. We are now in possession of a large amount of experimental data confirmatory of this view. An apparent objection to the conception of fermentations as accelerated reactions is contained in the fact that a single substance will yield different products under the influence of different ferments. The fact is however capable of another interpretation, one harmonious to the theory, that the product represents a stage in the reaction, and that different ferments accelerate to different stages. Secondary reac-

tions must also be considered. Recently, however, van't Hoff[1] and Wegscheider have pointed out that in special instances the addition of a positive catalysor to a system in a state of chemical rest may inaugurate a reaction; and it is possible that this may be of not such infrequent occurrence in the domain of organic substances. More detailed reference shall be made to this later. For all the known fermentations occurring in nature it is clear that the fermentations represent accelerations of existing reactions. These auto-reactions progress as a rule at ordinary temperatures with extreme slowness. In many instances however they have been studied and measured. This is true particularly for the auto-oxidations occurring in metals and the auto-hydrolyses noted in solutions of organic substances. We possess likewise accurate studies upon such reactions between gases. Indeed Victor Meyer's studies upon the slow reaction between gaseous oxygen and hydrogen at ordinary temperature were among the earliest investigations tending to indicate that catalyses are accelerated reactions, and not reactions de novo.

CHEMICAL REACTIONS OF FERMENTATIONS.

Fermentations are best studied and classified on the basis of the reactions involved, as long ago suggested by Henninger. This plan is simple, can lead to no confusion, demands no preconceptions, and places the trend of investigations in harmony with the general customs of the mother science. For every chemical phenomenon one may enquire: What is the reaction? and also: How and under what conditions does the reaction proceed? There are excellent illustrations of physico-chemical studies of chemical processes of which the reactions are entirely unknown. The researches of Arrhenius, the measurements of the relations between specific biological bodies and their anti-bodies, is an illustration in point. For the ferments, however, we possess actually more information upon the chemical nature of the reactions than upon the laws under which they proceed. The great difficulty in the past has been that so much of the work has been concerned neither with the nature of the reaction nor with the question of the physico-chemical relations, the studies have been conducted along vital lines, so that the results are not

adapted to purposes of exact interpretation. The study of a fermentation ought to be carried out from the double point of view of organic and physical chemistry; the neglect of these studies by the organic chemist and the narrow formal treatment of the problem by the physical chemist have been of detriment to the advance of our knowledge.

The most simple reactions of catalysis are the intramolecular rearrangements. Many such instances are met with in organic substances. Aldehyde on standing passes into the paraldehyde. The transformation proceeds more rapidly at higher temperature, and is especially accelerated by the presence of acids. Thus for acetaldehyde (Turbaba):

$$CH_3.CHO + CH_3.CHO + CH_3.CHO \leftrightharpoons CH_3 - CH\begin{matrix} O - CH - CH_3 \\ \\ O - CH - CH_3 \end{matrix}\!\!\begin{matrix} \\ O \\ \\ \end{matrix}$$

The polymerization into paraldehyde may be regarded as due to the conversion of the aldehdye $CH_3.CH=O$ into $CH_3.CH\begin{matrix} O- \\ \\ \end{matrix}$, following which three of such groups join in a ring structure to form a saturated substance. The modus of the action of the hydrogen ions in the acceleration of the polymerization is not known. The reaction is reversible, and tends to an equilibrium.

A very illustrative reaction of this type is the reversible formation of dianthracine from anthracine, discovered by Fritsch and recently studied by Luther and Weigert. This is a photochemic reaction, and tends to an equilibrium. The reaction of light is reversed in the dark, and thus the reaction is to be written $2\ C_{14}H_{10} \underset{\text{darkness}}{\overset{\text{light}}{\rightleftharpoons}} C_{28}H_{20}$. In all probability such photochemic reactions are very numerous. Nernst has indeed advanced the hypothesis that the formation of ozone from atmospheric oxygen is such a process.

Acetone slowly undergoes polymerization into di-aceton alcohol (Koelichen). The transformation is much accelerated by alkalies.

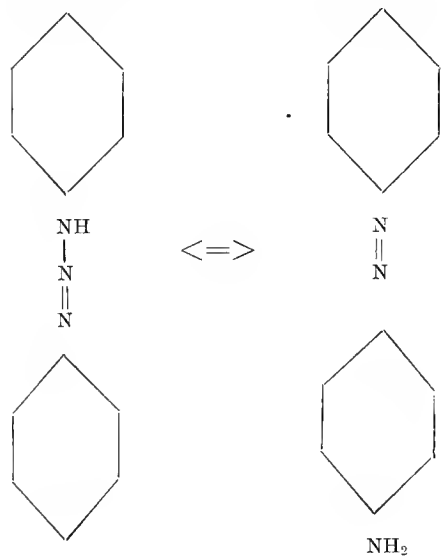
$$2\ CH_3.CO.CH_3 \leftrightharpoons (CH_3.CO.CH_3)_2.$$

$$CH_3.CO.CH_3 + CH_3.CO.CH_3 \leftrightharpoons (CH_3)_2C\begin{matrix} OH \\ \\ \end{matrix}\!\!-\!\!-\!\!-\!\!-CH_2.CO.CH_3.$$

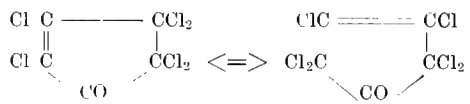
The reaction is reversible.

An excellent illustration for the aromatic series is afforded by the transformation of diazo-amido-benzol into p-amido-azo-benzol. Acids accelerate (Goldschmidt).

$C_6H_5.N:N.NH.C_6H_5 \quad <=> \quad C_6H_5.N:N.C_6H_4.NH_2$



An exceedingly pretty illustration of a catalytic intramolecular rearrangement is seen in the isomeric ketones C_5Cl_6O studies by Zincke and Kuester.



The reaction is reversible, and no matter from which ketone one proceeds an equilibrium will be established in the system. Though the sole difference lies in the positions of the double chlorines, the two isomers present melting points as different as 31° and 92°.

Fermentations of this type are not yet known.

In the next group of catalyses one substance is broken into two or more molecules, or for the reversed reaction two or more molecules combine to form one substance. Some of these reac-

tions are of the simplest of association and dissociation. Such are:

Hydrogen + oxygen <=> water.
$2\ H_2 + O_2 <=> 2\ H_2O$.

Hydrogen + iodine <=> hydriodic acid.
$H_2 + I_2 <=> 2\ HI$.

Sulphur dioxide + oxygen <=> sulphur trioxide.
$2\ SO_2 + O_2 <=> 2\ SO_3$.

These are all greatly influenced by changes in temperature, are typically reversible, and are markedly accelerated by the members of the platinum group. Another good illustration, studied by van't Hoff, is the following:

Dibrom-succinic acid <=> bromo-maleic acid + hydrobromic acid.
COOH.CHBr.CHBr.COOH <=> COOH.CBr.CH.COOH. + HBr.

Many fermentations are of this order.

d-glucose <=> aethyl alcohol + carbon dioxide.
$CH_2OH.(CHOH)_4.COH <=> 2\ CH_3.CH_2(OH) + 2\ CO_2$.

Malic acid <=> lactic acid + carbon dioxide.
$COOH.CH_2.CH(OH).COOH. <=> CH_3.CH(OH).COOH + CO_2$.

The acceleration of the reaction in the case of d-glucose may be accomplished by alkali or platinum, furnishing a good illustration of the identical natures of catalytic and fermentative acceleration. d-glucose is subject to other fermentations:

d-glucose <=> two mol. of lactic acid.
$CH_2OH.(CHOH)_4.COH <=> 2\ CH_3.CH(OH).COOH$.

d-glucose <=> butyric acid + 2 carbon dioxide + hydrogen.
$CH_2OH.(CHOH)_4.COH <=> CH_3.CH_2.CH_2.COOH + 2\ CO_2 + H_2$.

Lactic acid is the intermediary product.

So far as we know, these different products of the fermentation of d-glucose are not interchangeable, but are specific to the particular ferment.

The fermentation of sinigrin by myrosin is possibly the most complicated of the known reactions of this group.

Sinigrin <=> allyl-sulphocyanide + d-glucose + acid pot. sulphate.

$C_{10}H_{18}NKS_2O_{10} <=> C_3H_5.SCN + C_6H_{12}O_6 + KHSO_4$.

Especially noteworthy is the formation of an inorganic electrolyte as one of the products.

Most of the ordinary fermentations involve four or more component molecules. The substrate combines with another molecule, and then divides to form two or more molecules. That our point of view begins with the body to be fermented is simply a result of historical development and of common experience with the phenomenon. Dynamically the two processes in the equation are of the same dignity. We use the term substrate to indicate the substance that in the ordinary sense of the term is the main component in the reaction, the substance to be fermented. The term product is applied to the substances that result from the reaction. Dynamically, what would be the products of the reaction in the one direction are the primary substances with the reaction in the other direction. Similarly the second body in the ordinarily primary reaction, the substance that is added to the substrate, is not in common usage accorded the same dignity given to the substrate; but dynamically it is upon the same plane. For example: ester + water = alcohol + fatty acid. We term the ester the substrate, the alcohol and fatty acid the products. But if we mix the alcohol and fatty acid, ester will be formed, and under such circumstances the ester is the product. Now since both reactions are always taking place side by side in the system, the use of the terms substrate and products is simply a matter of convenience, based upon the fact that experiments at direct fermentation are common, while experiments at reversion are rare. The water of course is as essential a component in the reaction as the ester, and in the reversed reaction the water is as truly a product. Of these fermentations there are three large groups: hydrolyses, oxidations, and reductions.

The *hydrolytic cleavages* are extremely common. In these reactions water is added, and then the product of the union is divided into two or more molecules of one substance, or into two or more substances. In the reversions of these cleavage reactions, two or more molecules combine to form a larger molecule, with the extrusion of water. Hydrolytic cleavage seems to be universal in the world of organic substances; whenever these sub-

stances are dissolved in water, slow hydrolytic cleavages are inaugurated. These auto-hydrolyses have been demonstrated in a large number of instances. The thermodynamic demonstration of the nature of the phenomenon is contained in the fact that steam is a universal hydrolysing agent for these substances, and since the reaction occurs rapidly at high temperatures it must occur to some extent at low temperatures. The agent in the auto-hydrolysis may be confidently assumed to be the hydrogen or hydroxyl ion of the dissociated water. A further proof of the occurrence of these auto-hydrolyses is contained in the fact that hydrogen ions are general accelerators of these reactions with these substances. In a certain number of these reactions, reversions have been demonstrated, both by catalytic and enzymic agents; and the occurrence of such reversions under appropriate conditions is postulated for all.

Of these hydrolyses many illustrations may be given. Thus:

Cellulose + water $\rightleftharpoons$ hexose + hexose.

$$n\ (C_6H_{10}O_5)_n + n\ H_2O \rightleftharpoons n\ C_6H_{12}C_6 + n\ C_6H_{12}O_6.$$

The hexose is d-glucose. The same reaction is noted for glycogen. The common bacterial fermentation of cellulose yields no sugar, but only gases and acetic and butyric acid (Omelianski), carbon dioxide being evolved in all cases, but otherwise either hydrogen or methane. For starch and inulin similar relations hold.

$$n\ (C_6H_{10}O_5)_n + n\ H_2O \rightleftharpoons n\ C_6H_{12}O_6.$$

For starch the products are d-glucose when acids are employed as the catalysor, maltose when diastatic ferment is employed; from inulin only laevulose is secured. In the acid hydrolysis of starch the process passes through the stage of maltose and ends with the formation of the hexose; in the diastatic fermentation the process stops at the stage of the disaccharide. In all of these hydrolyses of polysaccharides, the reactions pass through many substages.

The disaccharides undergo similar cleavages, termed inversions. These follow the general type:

Disaccharide + water $\rightleftharpoons$ hexose + hexose.

$$(C_{12}H_{22}O_{11}) + H_2O \rightleftharpoons C_6H_{12}O_6 + C_6H_{12}O_6.$$

In the case of sacchrose the products are d-glucose and d-laevulose; maltose yields only d-glucose; lactose yields d-glucose and d-galactose, and rafinnose yields d-glucose, d-galactose and d-laevulose. These reactions appear to occur directly, in the ordinary sense of the term.

Closely related to the inversions of the disaccharides are the hydrolytic cleavages of the glucosides. Glucosides are combinations of a hexose, not with another hexose as in the case of a disaccharide, but usually with an aromatic substance, an alcohol or an aldehyde. Thus their hydrolyses follow the type of the inversions. The general type may be illustrated by helicin.

Helicin + water <=> salicylic aldehyde + d-glucose.
$C_{13}H_{16}O_7 + H_2O \leftrightharpoons OH.C_6H_4.CHO + C_6H_{12}O_6$.

There is a wide range of variety in the second components of these compounds. Thus arbutin yields hydroquinon; phloridzin, phloretin; tannin, gallic acid; gaultherin, methylsalicylic acid; while amygdaline yields, in addition to d-glucose, hydrocyanic acid and benzoic aldehyde.

For many of these hydrolyses of poly- and disaccharides autohydrolysis has been demonstrated directly. For all of these reactions hydrogen ions act as positive catalysors. For some of them the colloidal metals of the platinum group have been shown to act as accelerators. Ferments of the cytase type accelerate the hydrolysis of cellulose, ferments of the diastase type act positively for the group of polysaccharides, and enzymes of the type of invertase accelerate the cleavage of the disaccharides and glucosides. It is a noteworthy fact that the fermentation of polysaccharides, disaccharides and glucosides is an act of hydrolysis; the fermentations of the hexoses is not an act of hydrolysis. There are possibly exceptions to this rule; thus there is presumed to be a direct fermentation of lactose into lactic acid; but as a rule the fermentation of the higher sugars is a hydrolytic cleavage, while the fermentation of the primary sugars is a direct intramolecular cleavage. Hydroxyl ions are more prominent as a catalysor of the reactions of the primary sugars than of those of the poly-saccharides.

The cleavages of albuminous substances, that we term digestion, are all hydrolyses. The general reaction runs:

Protein + water <=> amido acids + amido acids.

There are many substages in the process. The end products comprise quite a number of different amido acids. These hydrolyses are also accelerated by hydrogen ions, and to some extent by colloidal platinum. The auto-hydrolysis has been experimentally demonstrated for several members of the protein group. Cleavage with steam was indeed one of the oldest methods employed for obtaining products of protein hydrolysis. None of these reactions have been reversed.

The fermentations of the fats are likewise instances of hydrolytic cleavage. All esters, both the synthetic esters and the natural fats, are hydrolyzed according to the general equations:

Aethyl acetate + water <=> aethyl alcohol + acetic acid (Ostwald, Wys, Knoblauch).

$CH_3.CO.O.CH_3.CH_2 + H_2O <=> CH_3.CH_2.OH + CH_3COOH.$

Olein triglyceride + water <=> oleic acid + glycerine.

$C_3H_5(C_{18}H_{33}O_2)_3 + 3H_2O <=> 3\ C_{18}H_{34}O_2 + CH_2(OH).CH(OH).CH_2(OH).$

These reactions are typically and measurably reversible. The reversibility of the fat splitting enzyme has also been demonstrated for several fats. The simple reactions are greatly accelerated by hydrogen ions, and to some extent by the colloidal metals of the platinum group. In many respects esters present the best opportunities for the study of ferment action.

It is thus seen that the fermentation of the members of the three great groups of foods—protein, carbohydrate and fat—are instances of the enzymic acceleration of hydrolytic cleavages. These hydrolyses are, in the conditions in which they occur in nature as well as under the circumstances of experiments, monomolecular reactions, at least in so far as the reaction in the direction of the right (the hydrolysis of the substrate) is concerned. The water of solution is so much greater than the water of combination, that the mass of the water in the system is for practical purposes constant during the duration of the reaction.

Other interesting instances of fermentative hydrolyses may be described.

Urea + water <=> ammonia + carbon dioxide (Fawsitt).
$CO(NH_2)_2 + H_2O <=> 2\ NH_3 + CO_2$.

Hippuric acid + water <=> glycocoll + benzoic acid (Schmiedeberg).
$C_6H_5.CO.NH.CH_2.COOH + H_2O <=> CH_2.OH.COOH + C_6H_5.COOH$.

Arginine + water <=> urea + ornithin (Kossel).
$NH_2.C:(NH)_2.(CH_2)_3.CH.NH_2.COOH + H_2O <=> CO(NH_2)_2 + CH_2(NH_2).(CH_2)_2CH(NH_2).COOH$.

These fermentations are accomplished by ferments contained in the liver. The cleavages may be accomplished as readily by the action of acids as by ferments. Interesting are the fermentations of the salts of the vegetable acids. A good illustration is afforded by calcium formiate (Hoppe-Seyler).[1]

Calcium formiate + water <=> calcium carbonate + carbon dioxide + hydrogen.

$$\begin{matrix}HCOO\\HCOO\end{matrix}\!\!>Ca + H_2O = CaCO_3 + CO_2 + 2\ H_2.$$

The hydrogen acts as an anti-catalysor.

Catalytic accelerations of hydrolyses are exceedingly common, not only in natural substances, but also in synthetic substances. Thus:

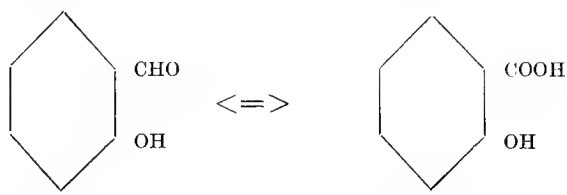
Mon-chlor-acetic acid + water <=> glycolic acid + hydrochloric acid (van't Hoff).[2]
$CH_2.Cl.COOH + H_2O <=> CH_2.OH.COOH + HCl$.

Under *oxidation fermentations* we understand such accelerations in oxidation as occur under the influence of the presence of a ferment. The steps in these oxidations are not well understood. It is not even known that oxygen is always added in these reactions, since oxidation can be effected by the withdrawal of hydrogen as well as by the addition of oxygen. Biologists have been inclined to group the fermentative oxidations under two headings, direct and indirect, according to whether hydrogen peroxide acted as a carrier, or not. The data are not sufficient to justify such a distinction. We ought not to dogmatize upon the nature of these reactions, since we are acquainted with

so few. When the knowledge of the auto-oxidation of inorganic substances now being accumulated (and which has been recently summarized from different aspects of the subject in the publications of Engler and Weissberg and of Kastle and Loewenhart) is applied to the study of these fermentations, we may expect light to break upon the subject. The best known instances divide themselves in two groups: those in which a substance combines with oxygen to form a single product; and those in which water is split off. Obviously the reversion of the latter would constitute hydrolyses.

Salicylic aldehyde + oxygen <=> salicycle acid (Jacquet).

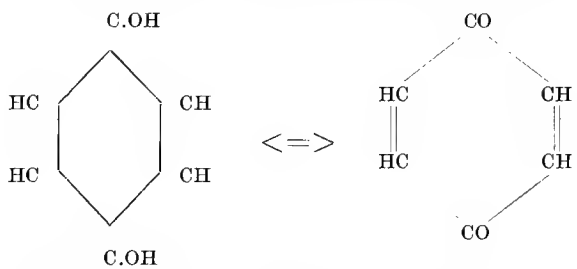
$2\ OH.C_6H_4.CHO + O_2 <=> 2\ OH.C_6H_4.COOH.$



A soluble enzyme of mammalian tissues accelerates this reaction.

Hydroquinone + oxygen <=> quinone + water (Bertrand).[1]

$2\ OH.C_6H_4.OH + O_2 <=> 2\ CO.C_4H_4.CO + 2\ H_2O.$



This fermentation, which is induced by the juice of the Japanese lac tree (laccase), and also by mammalian intestinal secretions, comprises, according to the modern conception of these substances, the transformation of a true aromatic substance into an alicyclic substance.

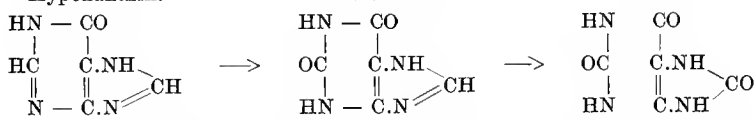
A good illustration is furnished by the acetic acid fermentation of aethyl alcohol, occurring in two stages.

Aethyl alcohol + oxygen <=> acetaldehyde + water.
$2\ CH_3CH_2.OH + O_2 <=> 2\ CH_3.COH + 2\ H_2O.$

acetaldehyde + oxygen <=> acetic acid.
$2\ CH_3.COH + O_2 <=> 2\ CH_3.COOH.$

According to recent investigations, oxidation ferments are very active in the purin catabolism. One illustration will suffice. Xanthinoxydase converts hypothanthin first into xanthin, then into uric acid. (Spitzer, Wiener, Burian).

Hypoxanthin. Xanthin. Uric acid.



Two interesting fermentations are brought about by the bac. xylinum (Bertrand).[2]

Sorbite + oxygen <=> sorbose + water.
$2\ C_6H_{14}O_6 + O_2 <=> 2\ C_6H_{12}O_6 + 2\ H_2O.$

Glycerine + oxygen <=> di-oxy-acetone + water.
$2\ CH_2(OH).CH\ (OH).CH_2(OH) + O_2 <=> 2\ CH_2(OH).CO.CH_2(OH) + 2\ H_2O.$

These reactions have not been reversed. For all of them inorganic accelerators are known.

While inorganic accelerators of the oxidation of the inorganic salts of metals are numerous, fermentative acceleration of such oxidations are known certainly to exist only for the nitrites. The reaction:

$$Metal — NO_2 + O <=> Metal — NO_3$$

is a reversible reaction that is going on constantly in soils and waters under the influence of bacteria, certain microorganisms being especially active in the one or the other direction. Whether in the reduction hydrogen is added and then water extruded is not known; indeed the mechanisms of the reactions have not been determined on account of the complexity of the conditions under which the phenomena occur. The oxidation of nitrite to nitrate has been long known to agriculture; the reduction of nitrate to nitrite has been learned more recently. Analogous fermentations have been described for the salts of sulphur, but the experiments lack confirmation.

Closely related to the simple oxidations are the reactions with hydrogen peroxide. Hydrogen peroxide tends to a slow reduction, and the oxidations of substances by hydrogen peroxide are in general to be regarded as accelerations of this auto-reduction.

Hydriodic acid + hydrogen peroxide <=> iodine + water (Brode).

$2\ HI + H_2O_2 <=> I_2 + 2\ H_2O.$

Sulphurous acid + hydrogen peroxide <=> sulphuric acid + water.

$H_2SO_3 + H_2O_2 <=> H_2SO_4 + H_2O.$

The oxidation of formic aldehyde occurs in two stages:

Formaldehyde + hydrogen peroxide <=> formic acid + water.

$H.COH + H_2O_2 <=> H.COOH + H_2O.$

Formic acid + hydrogen peroxide <=> carbon dioxide + water.

$H.COOH + H_2O_2 <=> CO_2 + 2\ H_2O.$

There is some evidence tending to indicate that the reversion of these reactions represents the initial steps whereby carbohydrates are formed by plants, though the reactions are usually written with oxygen alone (in the form proposed by Baeyer and Erlenmeyer): $CO_2 + H_2O = H.COOH + O$ and $H.COOH = H.COH + O$. The theory assumes the presence in the chlorofyl-containing cell of some enzyme accelerating the reductions, subject to the influence of the light. Since carbon dioxide and water are universally present in the atmosphere, one has but to assume the removal or combination of the formaldehyde (*i.e.*, its condensation into sugar) in order to possess a firm physico-chemical basis for the continued reduction. The influence of light has recently been interpreted by Euler[1] to lie in a translocation of the station of equilibrium in the direction of the reaction of reduction. Loeb[1] believes his studies indicate that in the reduction of carbon dioxide to formic acid, carbon monoxide is formed, and that both ozone and hydrogen peroxide appear. He suggests the following reactions:

$2\ CO_2 <=> 2\ CO + O_2.$

$CO + H_2O <=> H.COOH.$

$3\ O_2 <=> 2\ O_3.$

$O_3 + H_2O <=> H_2O_2 + O_2.$

Following which we would have

$CO_2 + H_2O_2 <=> H.CHO + O_2.$

From formaldehyde sugar would be formed by condensation. The steps in the earlier stages of the condensation of formaldehyde are not known; it is only certain that amido acids are not concerned (Euler).[2] This general conception has been given a certain experimental foundation in the investigations of Loeb[2] on the effects of the silent electrical discharge in a system containing carbon dioxide and water; reduction products were obtained that conform quite closely to the above scheme.

Though a certain amount of experimental work lies at the basis of these theories, it is apparent that the analytical demonstrations of traces of hydrogen peroxide and formaldehyde, appearing as transient stages in a reaction, must be a hazardous test. A further biological difficulty lies in the great toxicity of formaldehyde, though this could be obviated by the assumption of immediate combination or elaboration into higher carbohydrates.

The reactions of hydrogen peroxide are susceptible of acceleration by a large number of inorganic substances, especially by ferrous salts, colloidal metals of the platinum group, molybdic and tungstic acids. The acceleration of the reactions of formaldehyde and formic acid may be accomplished with plant extracts Bach has described the acceleration of the reduction of carbon dioxide to formic acid by uranium salts, a statement that Euler was not able to confirm.

Fermentative reductions, that is, the acceleration of reactions of reduction, have not been long known. Indeed Hoppe-Seyler was inclined to deny the existence of such fermentations. Accelerations by inorganic catalysors are extremely common. Curiously enough, our present knowledge includes few instances of the fermentative acceleration of reductions of organic substances, most of the recognized reactions concerning metallic salts. The reduction of the nitrate to the nitrite, mentioned in the previous paragraphs, is probably the most widely occurring reaction of the type known. This fermentation occurs also in the mammalian juices (Abelous). Very interesting are the reductions of the acids and salts of selenium and tellurium by certain bacteria described by Gosio. These reductions follow the regular types:

Selenous acid + sulphurous acid <=> sulphuric acid + selenium + water.

$H_2SeO_3 + 2\ H_2SO_3 <=> 2\ H_2SO_4 + Se + H_2O.$

Tellurous acid + sulphurous acid <=> sulphuric acid + tellurium + water.

$H_2TeO_3 + 2\ H_2SO_3 <=> 2\ H_2SO_4 + Te + H_2O.$

We do not know what the reducing body that reacts with the selenous and tellurous acids (replacing the sulphurous acid in the written reactions) in these bacterial experiments actually is, but the acceleration is very pronounced, and as stated is observed with the -ous and -ic acids and their salts alike. In some instances, the reduction may be only to a lower oxide.

Similar reactions occur with arsenic, both in the arsenous and arsenic states.

Arsenic trioxide + hydrogen <=> arsenuretted hydrogen + oxygen.

$2\ AsO_3 + 3\ H_2 <=> 2\ AsH_3 + 3\ O_2.$

Arsenic trioxide + potassium acetate <=> kakodyl + pot. carbonate + carbon dioxide.

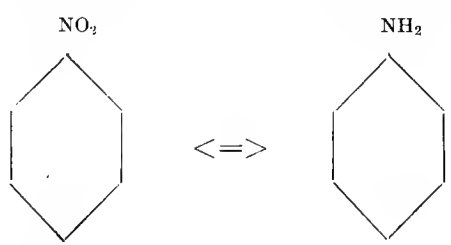
$As_2O_3 + 4\ KC_2H_3O_2 <=> (As(CH_3)_2)_2O + 2\ K_2CO_3 + 2\ CO_2.$

These accelerations are produced in the culture media of certain bacteria; the steps and details in the reactions are not worked out.

Of the fermentative reductions of organic substances we will cite two:

Nitrobenzol + hydrogen <=> anilin. + water (Abelous and Gérard).

$C_6H_5.NO_2 + 3\ H_2 \quad <=> \quad C_6H_5.NH_2 + 2H_2O$



This reduction is accelerated by some substance contained in extracts of mammalian tissues and in extract of yeast. What

the actual reducing component in the reaction is, we do not know; it is certainly not hydrogen itself.

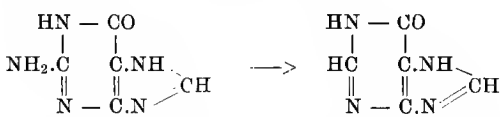
Aspartic acid + hydrogen <=> ammonium succinate (Hoppe-Seyler).[2]

$COOH.CH(NH_2).CH_2.COOH + H_2 <=> COOH.CH_2.CH_2.COOH.NH_3$.

This reduction is accomplished by many of the common bacteria, such as the Bacillus coli communis. The component substance that reacts with the amido-succinic acid is not known.

Other ferments are known that do not fit naturally into any of these groups. An illustration may be given in the desamidation ferments, substances that accelerate the replacement of the amido group by an hydrolyl group in the various amido acids that are products of the hydrolysis of protein, and also the replacement of the amido group in guanine and adenine by hydrogen. Thus the action of guanase (Jones) may be illustrated as follows:

Guanine + H_2 = hypoxanthin + NH_3



These conversions are in the broad sense oxidations, just as the removal of a CO_2 group constitutes a reduction.

These illustrations will suffice to afford a cursory view of the final relations determined by the reactions of fermentation of different types.

For nearly all of these accelerated reactions the slow autoreactions are known. For many of these reactions reversions have been accomplished. For many of these reactions the influence of increases in temperature is known. These facts afford a natural presumption that the reactions of catalysis and fermentation are essentially identical.

In looking over these reactions one cannot fail to be impressed with certain general features. The heat relations in fermentations vary. In the common hydrolyses the products have approximately the same heat values as the substrate. The oxidations on the contrary are exothermic, the reductions endo-

thermic reactions. Natural fermentations convert complex substances into simple substances, they convert colloids into electrolytes, electrolytes into simple gases and elements. Most of the substances that are commonly the substrates of fermentations are but slightly soluble in water, or even incapable of true solution but only of colloidal suspension. Starches, cellulose, proteins, glucosides, fats, all form in water more or less colloidal suspensions, and display further the tendency to form hydrogels. When these substances are fermented, the products are substances of greater solubility, quite devoid of colloidal characteristics, with no tendency to the formation of hydrogels. Through the fermentation the system has been converted from a heterogeneous to a homogeneous state. The substrates of natural fermentations are usually substances of very large molecular weight, the products possess small molecular weight. These substrates are almost devoid of the power of diffusion, the products diffuse readily as a rule. The natural substrates exert but little depression of the freezing point of solutions, the products exert a marked depression as a rule; the substrates possess little osmotic pressure, the products marked osmotic pressure. The substrates are rarely crystalline, the products are usually crystalline. The substrates are substances that are not subject to electrolytic dissociation. The products exhibit this property. The substrates are substances with little tendency to chemical reaction as compared to the products. There are of course exceptions to these statements; for instance, the properties of the higher fatty acids are in these regards but little different from their fats. The natural fermentations usually comprise the disintegration of complex substances that have been synthesized in the vegetable or animal organism. Since all fermentations are to be regarded as reversible processes, how obvious is the suggestion, first clearly formulated by van't Hoff, that the natural fermentations are simply the reversions of the reactions whereby these substances were formed. As we shall see, there are no thermodynamic reasons that plead against this proposition. The fact that fermentations of the natural order are exothermic reactions has been employed by some biologists in support of a teleological interpretation of the circumstances, according to which a syn-

thesis could not be held to be accomplished by the action of a ferment. As shall be pointed out, this interpretation is lacking in theoretical validity.

It must be conceded that the pendulum may swing too far in the opposite direction, and that fermentations may be accorded a too general scope in physiological and pathological processes. The theoretical possibility for such an over-generalization lies in the very definition of fermentation. We have defined fermentation as the acceleration of some existing reaction by a substance formed in a vegetable or animal organism. When we reflect further that theoretically every reaction is capable of acceleration, the boundless application of the principle becomes apparent. It is, however, clear that chemical biology is in no greater danger of becoming simply a treatise on fermentations, than are inorganic and organic chemistry in danger of becoming reduced to the boundaries of catalysis, since the same principles apply to each. In each individual instance the question whether a particular phenomenon may be a fermentation (or a catalysis) is plainly a matter of concrete demonstration. And it is because this concrete demonstration must be so much more difficult in biological than in chemical questions, that there will be a tendency among biologists to overwork the principle. Standing bewildered before the apparently hopeless complexity of a biological problem, it is so easy to say "ferment action." This very tendency compels us to insist with the greatest objective strictness upon the precise demonstration of the occurrence of a fermentation. The safety of the investigator lies in close adherence to the laws of general chemistry connected with the kinetics of reactions. Not only do these control us in our studies, but they enable us to investigate in a proper and adequate manner the characteristics of a fermentation, after its identity as such has been established. The importance of the study of fermentations has been entirely underestimated by the biological world. While in the groupings of systematic biology it may have sufficed to know simply that a particular phenomenon was a fermentation, for the real study of the chemistry of the functions of animals and plants, that is simply the stating of the problem. The height of biological research is the reproduction of an act of

nature. To attempt to reproduce the chemical functions of organized bodies, one must study fermentations from the point of view of the control of its several variables.

Within recent years the biological relations of the ferments have been investigated. That there are natural protective influences against the action of ferments is known, and certain native forms of protein, for example, seem almost entirely resistant to the action of proteolytic ferments. Artificial immunity to the action of a particular ferment has been described as being established as the result of the reaction of an animal to injections of the ferment, and the term anti-ferment has been given to the hypothetical substance endowed with this property. Interesting as these observations are, so far as known they do not bear any relations to the problem of ferment action, and we shall give them no further consideration.

APPLICATION OF LAWS OF CATALYSIS TO FERMENTATIONS.

We define a catalysis as an acceleration of an already existing reaction through the presence of another body that does not appear in the end-products of the reaction. In the specific instance there are two criteria of a catalysis. Every alteration in velocity not dependent upon alteration in concentration or of temperature indicates catalysis; and in the catalytic acceleration there are no stoichiometric relations between the catalysor and substrate or products. There is theoretically a catalysor for every reaction, and every substance may act as a catalysor. Certain classes of bodies possess to a high degree this quality of acceleration: the platinum group, all colloidal metals, hydrogen and hydroyl ions, and the oxides and oxyhydrates of the elements of varying valency, such as iron, manganese, and nitrogen. The relations of energy involved in a reaction are not disturbed by a catalytic acceleration; the result is achieved by a diminution of the chemical resistance. The existence of a large class of compounds in the natural state is dependent absolutely upon their chemical resistance, and were this materially diminshed, these compounds would cease to exist. Chemical resistance diminishes with increasing temperature, and there is for each concentration of a chemical system an optimal temperature. Cor-

responding to the physical state of the system, we distinguish between catalyses in homogeneous and in heterogeneous systems. The current theory of physical states, well formulated by Spring, is that from the homogeneous to the heterogeneous state is a gradual transition. In fermentations we deal with bodies that present with water less of homogeneity than solutions of pure crystalloids, and usually less of heterogeneity than the typical colloids. We shall see that the behavior of fermentations confirms this interpretation.

In our consideration of the kinetics of catalysis we are concerned in the first instance with the law of mass action and especially as applied to a reaction of which the products reunite to form the original substance to a measurable degree; and secondly with the relations observed when such a reaction is accelerated by the presence of a positive catalysor or enzyme. Theoretically all reactions are to be looked upon as limited reactions and likewise reversible; but the point of equilibrium may be so slightly removed from the condition of a complete reaction as not to be analytically determinable; and it is not always possible to fix the conditions favorable to a reversion so that the theoretical result shall become experimentally apparent. What is about to be enunciated under these headings is specifically valid only for homogeneous systems; the relations involved when heterogeneous systems are concerned will be considered later.

The fundamental proposition concerned in the kinetics of chemical reactions, as early formulated by Wilhelmy, is that under constant conditions of experimentation the transformation in the unit of time is proportional to the mass of the reacting bodies. This proposition is directly analogous to the Newtonian law for the radiation of heat from a warm to a cool body; the radiation in time is proportional to the difference in temperature between the two bodies. The formulation may be expressed in another way in the statement that whenever a transformation is taking place, the rapidity of that transformation in a particular moment is proportional to the distance between the endpoint (the equilibrium) and the point in the course of the reaction attained at that particular moment. Applied to the concrete instance, say to the inversion of cane sugar, we mean that

in each particular moment the amount of sugar inverted is proportional to the amount of uninverted sugar present in that same moment. This applies as stated only to monomolecular reactions, reactions in which the mass of only one substance is affected during the reaction. But the general proposition applies to bimolecular and trimolecular reactions, with the difference that the degree of reaction in time depends upon the concentration of the two or three active masses, instead of depending simply upon one. Now mathematically all the fermentations occurring in the biological world are monomolecular reactions, because the second body concerned, as water or oxygen, is present in such excess that alteration in its mass is of almost no consequence. If, for instance, in the reaction cane sugar + water = glucose + fructose the amount of water present were approximate to the amount required in the reaction, that reaction would be treated as a bimolecular reaction; but as the experiment is carried out in dilute solution, where the water, as solvent, is present in a thousand times the amount required for the water as reagent, the reaction is equivalent to a monomolecular reaction. For this reason we will confine ourselves to the kinetics of the monomolecular reaction.

This proposition is expressed in the general differential equation $-\frac{dt}{dx} = C\,(A-x)$. A is the original amount of substrate, x the amount of substance converted in the time t, C is a constant. The equation holds only when the temperature and volume are held constant, and the system is in a state of certain dilution. When integrated and reduced to its simplest relations under the stipulation that when $t = 0$ x also $= 0$, the equation becomes $C = \frac{1}{t} \log \frac{A}{A-x}$. The coustant expresses the work done under constant conditions. If, for example, we determine with a particular strength of acid in an inversion experiment that $C = 0.002$, that means that under constant conditions of temperature, volume and concentration of acid, in a solution of sugar of the strength of a gramme-molecule in the liter, 0.002 gramme-mol. of sugar will be inverted in the first minute, and if we could add to the system in each minute without changing the volume 0.002 gramme-mol. of sugar, that quantity would be inverted regularly each minute.

This simple relation becomes more complicated when we deal with a reaction of active and measurable reversibility. Under these circumstances an equilibrium is established in the system when the opposing reactions just compensate each other. The reaction in the direction of the right becomes less each minute as the mass of substrate becomes less; the reaction in the direction of the left becomes greater each moment as the mass of the products of the other reaction increase. At a certain point these will be equal, and from this time no apparent change will be observable in the system. But it must not be supposed that the reactions have stopped; they continue as before proportional to the active masses of the respective components, but since they are balanced, the system is in equilibrium.

The reaction may be written in the following manner, using as an illustration the reaction ester + water = alcohol + fatty acid.

$$\text{Ester} + \text{water} \rightleftharpoons \text{alcohol} + \text{fatty acid}.$$

$$\text{Const.}_{\longrightarrow}\ \text{Concentrn.}^{\text{ester}} \cdot \text{Concentrn.}^{\text{water}} = \text{Const.}_{\longleftarrow}\ \text{Concentrn.}^{\text{alcohol}} \cdot \text{Concentrn}^{\text{fatty acid}}$$

$$\frac{\text{Concentrn.}^{\text{alcohol}} \cdot \text{Concentrn.}^{\text{fatty acid}}}{\text{Concentrn.}^{\text{ester}} \cdot \text{Concentrn.}^{\text{water}}} = \frac{\text{Const.}_{\longrightarrow}}{\text{Const.}_{\longleftarrow}} = CONST.$$

CONST. stands for the constant of equilibrium.

When a catalysor is added to systems representing each of these types of reaction, under conditions of controlled relations in experiments with pure substances, nothing happens except that the time of the reactions is shortened. The accelerated reaction that naturally was practically a complete reaction remains practically a complete reaction; it is simply completed more quickly; and with different quantities of catalysor the differences are simply those of degrees of rapidity. The accelerated reaction of measurable reversibility remains a reaction of reversibility and the point of equilibrium is not disturbed by the acceleration; the $\frac{c_{\longrightarrow}}{c_{\longleftarrow}} = C$ is simply reached more quickly. As I have stated however the conditions must be controlled. The concentrations and volumes must be held constant, in order that the relations of the active mass or masses are maintained; the

temperature must be held constant because the point of equilibrium may vary with the temperature; and the products must not combine with the catalysor, for in this manner also would the constant of equilibrium be disturbed. It is also very important that pure substances be employed, since traces of foreign bodies may exert a very great disturbance.

Let us now examine some of the conditions and variations in detail.

Initial Concentration of the substrate. This is not immaterial, and it is not true that at all concentrations of the substrate the reaction in any particular moment is proportional to the active mass of the substrate. This statement is true only of dilute solutions, just as the laws of electrolytic dissociation hold true only for dilute solutions. In the inversion of a dilute solution of sugar the law of Wilhelmy is obeyed; but in the inversion of a thick syrup, no such result is obtained. If one endeavors, in accordance with the current tendency, to rest catalytic reactions upon the theory of ionization, this fact becomes in a general sense intelligible. A further bearing upon the disturbing action of high concentrations of the substrate lies in the fact that under such circumstances the substrate participates in the role of solvent; the vapor pressure of the catalysor may be thereby altered, since under such circumstances the solvent will be modified. Not only is the velocity of a reaction dependent upon a proper dilution of the substrate; the order of a reaction is likewise altered by excessive concentration. In a monomolecular reaction, under conditions of proper concentration, when the system is diluted the times of equal proportional transformation are not altered; doubling the concentration doubles the velocity. Under similar circumstances with a bimolecular reaction, when the system is diluted the times of equal proportional transformation are inversely as the initial concentrations; doubling the concentration quadruples the reaction velocity. Now under conditions of high concentration these relations do not hold, and it is thus necessary to provide for proper dilution when determining the order of a reaction as well as when determining the velocity. The necessity for high dilution of the substrate is most urgent in the case of substrates of ponderous molecular weight.

Concentration of the products. This is likewise not immaterial. In general, the higher the concentration of the products, the more energetic the process of reversion. But in experimental catalysis it is sometimes found that when the concentration of the system is high the resultant high concentration of the products disturbs the course of the reaction.

Influence of temperature. The acceleration of reaction velocities by increase in temperature holds good for catalytic reactions as well as for simple ones. For such work the simple formula of Arrhenius will usually suffice: $ln \frac{k_1}{k_2} = A(\frac{T_1 - T_2}{T_1 \cdot T_2})$, A being the constant, and k_1 and k_2 the velocity constants of the reaction at the absolute temperatures T_1 and T_2. This equation has been tested on many catalytic reactions. Corresponding very closely in its results to the above equation is the empirical rule of van't Hoff that for every ten degrees increase in temperature the velocity of a reaction is doubled, *i.e.*, the time required to do a unit of transformation is reduced one-half, $\frac{V at\ T_{n+10}}{V at\ T_n} = 2+$. For ferments the rule holds good in a restricted sense. The rule holds for most ferments from about 15° to 35°; above this the increase in velocity is often much more than predicated by the rule, while above 45° there is a rapid fall to zero. The fall is the result of the destruction of the ferment, a condition not contemplated in the rule. As a whole however, in consideration of the complexities attending the experimentation, the concordance of the facts with the theory is quite good.

A moment's consideration of the thermodynamic relations concerned will indicate that the formulae could not be expected to hold good unless the catalysor remained unaltered and no secondary reactions intervened. These conditions are rarely present in a fermentation. The best evidence of this lies in the experimental fact that the temperature optimum is a very inconstant and shifting moment, and that the catalysor is inactivated in most fermentations. The conditions surrounding the experiment have a great deal to do with the temperature optimum; in some work with the digestion of protamine by trypsin, for example, I found that the presence of a little of the vulcanization powder from the inside of new rubber tubing would lower the temperature optimum several degrees. The principal

factor however lies in the reactions between the solvent and the catalysor.

It is a common error to suppose that a temperature optimum is peculiar to ferment action. Every reaction has a temperature optimum, and this optimum is usually altered (lowered) in a catalytic acceleration. The system $SO_2 + O = SO_3$ (Knietsch) has its temperature optimum about 900°; at 1000° the system is in equilibrium, the velocity of the reactions being however very slow. In the presence of platinum the temperature optimum is lowered to about 450°, the velocity is rapid, and as the reversed reaction (the dissociation of SO_3) does not occur to any material extent below 500°, the acceleration of the reaction is very effective. The catalytic acceleration of the formation of water from oxygen and hydrogen also displays a sharp optimum (Ernst). Ferments are notable simply for the lowness of the temperature at their optimum, and for the narrowness of the optimal range. The latter condition has lost much of its significance since we have learned that the sharp descent of the curve from the optimal temperature is due in large part to the destruction of the ferment.

Relations of the catalysor. The typical positive catalysor simply accelerates the velocity of a reaction, without altering the nature of the reaction, the order of the reaction, the character or yield of the products, the point of equilibrium, and without being itself altered in the process. It is just as though there were a certain resistance to the progress of the reaction, and the presence of the catalysor diminished this resistance. These conditions are usually maintained in inorganic catalyses, are as a rule not fully maintained in the acceleration of reactions involving organic substances, while they may be said never to be fully realized in fermentations. Since the presence of the catalysor simply accelerates the reaction, it ought in general to do so proportionally to the quantity of the catalysor employed, and this is usually true. Especially for the catalytic action of hydrogen ions has this relationship been shown to be true; the catalytic action of acids is related to their electrolytic dissociation. The same holds true for hydroxylions. For other catalysors the same rule has in general been found to hold good. There are however

several well studied reactions, for which the catalytic acceleration is not proportional to the quantity of catalysor. Thus Noyes has shown that in the reaction between stannous chloride and ferric chloride the catalytic acceleration of acids is proportional to the square of the hydrogen ions; and in the catalytic reduction of hydrogen peroxide by colloidal platinum the acceleration is not proportional to the quantity of platinum (Bredig). For ferments the relations have not been well studied, and most of the reported statements are not worthy of credence because of uncontrolled methods of experimentation.

The relation of the mass of ferment to the acceleration of the reaction may be usually expressed in the equation of Bredig.[1] $\frac{C_1}{C_2} = (\frac{F_1}{F_2})^m$. The C is the constant of velocity for the ferment concentration F, m is a constant. For the common catalysors, $m = 1$. According to Pawlow, m is ½ for ferments, that is, the so-called rule of Schuetz holds. With the more careful control and measurement of ferment reactions, however, m is more and more often being determined to be 1. The proportionality holds for low concentrations.

To the statement that the order of a reaction is not altered by the presence of a positive catalysor, exceptions seem to exist. The reaction $H_2O_2 + 2\ HI = 2\ H_2O + I_2$ depends upon the concentration of the two reacting bodies; that is, it is a bimolecular reaction. Brode studied the acceleration of the reaction under the influence of molybdic acid and iron sulphate, and found that under these circumstances the velocity of the reaction was a function of the concentration of hydrogen peroxide—that is, the presence of the catalysor had converted the reaction from one of the second to a reaction of the first order. There exist also other studies suggesting a similar shifting in the order of the reaction, though in none have the relations been so carefully worked out. The alteration is unquestionably to be explained upon the basis of intermediary reactions.

The statement that the station of equilibrium is not translocated by the presence of a catalysor seems to hold absolutely good unless the catalysor is altered in the progress of the reaction, and there is for this statement experimental evidence as well as thermodynamic theory. The acceleration of the catalysor

per se simply multiplies the $\frac{<}{c}$ and $\frac{}{c}$ of the equation, the C is not affected by the acceleration. When however the catalysor is altered during the course of the reaction, it means that the substrate, the products or the solvent has entered into reaction with the catalysor, and as a consequence the concentration of one or the other member of the system has been altered, and as a result of this the station of equilibrium has been shifted.

The remarks previously made upon the necessity of adequate dilution hold also for the catalysor. When to a system is added an excess of a catalysor, bizarre and irregular results may be expected. For such catalysors as act through ionization this behavior is quite intelligible. It is here again possible that at a high concentration of the catalysor, it will in part participate in the solution of the substrate. Under such circumstances the solvent is not identical with that in a system at high dilution, and since the station of equilibrium may be shifted very materially by alterations in the solvent, irregular results might be expected for this reason alone. Furthermore, the active mass of the substrate cannot be assumed to be the same in the changed state of the solvent. There is in any event no necessity for the use of high concentrations of the catalysor, since one of the most striking aspects of these accelerations is the minimal amount of catalysor required to effect a notable acceleration.

When to a system are added several catalysors, what happens? Their accelerations may be simply superadded, or the results may be greater or less than the sum of their individual reaction velocities. It is apparent that we have secondary reaction to deal with, through which alone the abnormalities are to be explained. In some instances the second catalysor is formed as a product during the course of the reaction, and the consequent increment is spoken of as auto-catalysis. A good illustration is afforded in the catalytic acceleration of the cleavage of aethyl acetate into aethyl alcohol and acetic acid. Let us suppose that the entire system be so diluted that the acid acting as a catalysor is entirely dissociated. Now as the acetic acid is set free, it will likewise undergo a certain dissociation, and the concentration of hydrogen ions in the solution will be thereby gradually increased; and since the acceleration is proportional to

the concentration of hydrogen ions, the velocity will increase. This increment is an auto-catalysis. Pure nitric acid attacks copper very slowly, but the reaction gradually becomes more rapid as more nitrous acid is formed in the reduction of the nitric acid; here nitrous acid, a product of the reaction between copper and nitric acid acts as the auto-catalysor.

Under the term inactivation are understood currently several different relations. The slowing of the reaction under the influence of concentrated products is a manifestation of the mass law, and ought not to be referred to as an inactivation of the ferment. The ferment may be inactivated by union with some extraneous body, or even by its mere presence; in a purely chemical inactivation, the ferment will be restored to power when the offending condition is removed. Lastly the ferment may be inactivated by hydrolysis or oxidation, in which it is destroyed as a catalytic agent.

Under certain conditions the presence of a substance not itself a catalysor will enhance the acceleration of a known catalysor. A good illustration of this fact is contained in the observation of Traube, that the presence of a trace of cupric sulphate, in itself inactive, will increase enormously the acceleration of the reduction of hydrogen peroxide by ferrous sulphate. The mechanism of such zymoexcitation is entirely unclear.

As there are accelerating substances, so there are retarding substances. We must here distinguish between negative catalysors and anti-catalysors. A negative catalysor would be a substance that retards the natural reaction, what might be called the auto-reaction. Anti-catalysors are substances that inhibit in part the accelerating action of a positive catalysor. Without denying that genuine negative catalysors exist, it is certain that the bodies described as such have been anti-catalysors. Titoff practically denies that true negative catalysors exist, and Young has drawn the same conclusion from his researches. In many inorganic reactions the most trifling amounts of foreign substances may act as pronounced anti-catalysors, and there can be no doubt that in the domain of ferments such influences are still more numerous and potent.

The *station of equilibrium* in a reaction of reversible character is dependent upon certain conditions. In the ideal sense it applies to a system at constant temperature in a high and constant dilution. It is possible experimentally to alter the station of equilibrium in several ways. More of the substrate may be added, in which event the reaction in the direction of the right will again assume the leadership until a new balance is established. Some of the substrate may be removed, in which event the reaction in the direction of the left will be accelerated until a new balance is established. The products may be removed or more of those bodies added, with the obvious resultant accelerations in the respective reactions until new equilibria are established. Or only one of the products may be added; thus one can add acetic acid to the system aethyl acetate + water = acetic acid + aethyl alcohol until the equilibrium is established in the sense that there are but two bodies present, aethyl acetate and acetic acid. The station of equilibrium may also be altered by dilution or concentration of the entire system. Alteration in temperature may also bring with it an alteration in the station of equilibrium, must indeed if the reaction be endo- or exothermic. To these we must add two further procedures for the translocation of the point of equilibrium—reactions between ferment and components, and reactions between solvent and product. These considerations of alterations in the station of the equilibrium are of great importance in the study of fermentations, and much of the reigning confusion has been due to neglect of them.

Of great influence is the *nature of the solvent,* the velocity of numerous reactions varying widely with different solvents. For the study of fermentations of biological origin this is of little consequence, since water is the invariable solvent. It is possible that the solvent in its relations to the concentration of the reacting bodies may be influenced by the presence of extraneous substances. We know that in very concentrated systems the substrate acts in part as solvent for the catalysor; and in high concentrations of the catalysor this body acts as a solvent for the substrate. In either event the point of equilibrium would be shifted.

Purity of the reacting bodies is generally of great importance for the regular progression of catalytic accelerations. The simple presence of foreign bodies is not itself of necessity a disturbing factor; extraneous substances may be divided into active and inactive. A trace of an active substance may seriously disturb the reaction in an otherwise flawless catalysis; a good illustration is afforded in the inhibition that may be exerted upon a large mass of platinum by a trace of arsenic, in the contact method for the manufacture of sulphuric acid. On the other hand, the mere presence of one or a number of inert substances may have little or no disturbing effect. We shall later see that it is sometimes possible to secure very good quantitative results in fermentation experiments conducted with materials known to be very complex. In other instances good results are not secured, the difference depending not upon the presence of foreign substances, but upon the presence of particular foreign substances that are active in their chemical relations to some member of the system under investigation. The figures obtained by Arrhenius in his studies upon the quantitative relations between biological bodies and anti-bodies present an excellent illustration of the fact that quantitative measurements may sometimes be secured under conditions of great complexity in the systems, provided only that the reactions under investigation be only sufficiently specific and elective. Nevertheless it were always best to work under pure conditions, and these would be, in the case of the subject occupying our attention, a pure substrate, dissolved in a pure solvent, and the reaction accelerated by a pure catalysor. The best studies in the literature have been accomplished under such conditions. In the various chemical investigations upon this matter it has been acids, alkalies and inorganic salts in particular that have produced disturbances in the progress of catalytic accelerations. A priori we can understand that since we conceive the modus operandi of catalytic acceleration to lie in successive intermediary reactions, there must be for each type or group of reactions certain substances whose introduction into the system would lead to different reactions that would proceed more or less at the expense of and certainly to the marked disturbance of quantitative progression in the original reactions. Just as

there are certain substances and conditions that prevent a good yield in the synthesis of organic substances, and these conditions and substances vary for the different synthetic procedures, so there are conditions and substances that disturb or prevent the regular march of a catalytic acceleration, and these conditions and substances will vary with the different reactions whose accelerations are being studied.

Furthermore irregularities may arise in the reactions when pure substances are employed. In many instances the reacting possibilities in a system are not limited to one reaction according to one formula, but may permit of reactions according to more than one formula. Under such circumstances, should the actual reaction follow other than the reaction according to one formula, the results would exhibit deviations more or less removed from the velocity demanded by the simple equation, and in proportion to the extent of the deviation from the single simple reaction. In other instances the products of a pure reaction themselves undergo reactions not tending to a reversion into the original substrate. Thus the products may react with each other, and then a totally new series of reactions will accompany the primary reaction. We see in the instance of the acceleration of the reaction $SO_2 + O = SO_3$ through the simultaneous presence of the reaction ferro-salt + O = ferri-salt, how the presence of an additional reaction may alter the reaction velocity; and such influence may be conceivably retardative as well as accelerating. Not only may the products react with each other; they may react with the substrate, with the production of an auto-catalysis or auto-anti-catalysis; or they may in other ways disturb the progress of the reaction, as by combining with the substrate, whereby the concentration of the active mass of the substrate would be altered. Strictly speaking, reversion of the reaction and autocatalysis are to be classified also as complicating factors that disturb the regular progress of a reaction in accordance with the general law of mass action, and systematic authors like Ostwald do so classify these conditions.

PRESENT APPLICATION OF KINETICS OF REACTIONS TO FERMENTATIONS.

When now we apply these considerations to the study of the natural ferments of the biological world we experience intellectual sensations that include both surprise and shock. The literature on the enzymes is enormous, and from the most varied points of view a most stupendous amount of work has been devoted to this subject. In so many instances however has the definition of the work been so uncontrolled and the execution so indefinite that the results have in no wise contributed to an actual elucidation of the relations concerned. Many of the difficulties however are almost inherent in the nature of the material. One cannot fail further to note in the biological investigations of fermentation an attitude of conscious or unconscious antagonism to the physical and chemical point of view in the problem. It must be realized that the biological and medical world has in large part, and up to within recent date, occupied a situation of constitutional inimicality to the physico-chemical interpretation of biological phenomena. This has been revealed in so many investigations of fermentations. The physicist, when investigating the validity of a law in a series of phenomena fixes the conditions of experimentations within such limits as have been indicated to contain the naturally favorable and controlled relations of the process under investigation. In much of the study of fermentations, on the contrary, the method of procedure has been to carry out the experiments not in the narrow zone of controlled conditions, but in the widest zone of uncontrolled conditions; and then, when the experimental results did not bear out the postulations of the law, to denounce the law.

Concentration of the substrate. This is in fermentations often difficult to adjust and determine. Most of the materials that are employed as the substratum of fermentations are natural substances, and therefore never pure. Furthermore it is often not possible to determine the quantitative degree of impurity. Let us take starch for illustration. Starch contains a certain amount of ash, composed of various inorganic bodies. It contains also organic bodies, traces of sugar, fats, protein and what not more. These bodies cannot be separated from starch,

and the only way of determining the actual amount of starch is to hydrolyse completely with strong acid and then determine the sugar present, and even this method would neglect the preformed sugars. When therefore one prepares a 1 per cent. solution of starch, one does not know with just how much under a 1 per cent. solution one is actually working. But more, starches vary in their resistance to hydrolytic cleavages, depending upon their origin, mode of preparation, age, and method of preservation. A certain starch might be already hydrolysed to as much as 10 per cent., according to the method of preparation and preservation. Let us further consider albumin. All that has been said of native starch holds true of native albumin to still greater degree. Not only are the foreign substances present to greater extent and in greater variety than in starch, but the tendency to alteration in the albumin itself is more pronounced. Furthermore an albumin is usually composed of several proteins, and unless one works with a pure protein like casein, the experiment actually involves the digestion of several proteins of possibly widely varying resistance to hydrolysis. While it is possible in some instances, as shown by Henri, to carry along in the same solution two catalyses of different materials and have each maintain its own proper velocity (the inversion of sugar and the cleavage of methyl acetate by acids), it would not be possible to do so under circumstances where the products of the one reaction make the measurement of the other reaction uncertain or futile. One could certainly not hydrolyze aethyl acetate and aethyl succinate in the same solution and secure anything but irregular results. Yet this is what is attempted every day when egg albumin is employed as the substrate. It is clear therefore that it is often not possible to fix the concentration of and to exclude a plurality in the substrate. Now since the concentration of the active mass of the substrate is a fixed requirement, it is clear that at the very outset in a fermentation we are confronted with an inability to define properly the conditions of the experiment. In the early part of the fermentation this indefinite minus in the concentration will not disturb the results so seriously, but towards the close of the reaction the influence must be marked. This state of affairs is of course not general; we can

prepare cane sugar, any of the hexoses, alcohols and the synthetic fats with a high degree of purity, and it has been along these lines that much of the best work has been done. One ought always to attempt the purification of the other materials, but very often such attempts at purification only lead to some denaturation of the substance, so that nothing is gained.

Experience with fermentations teaches that there are three zones of concentrations with particular behaviors. In the zone of high concentrations, variations lead to no changes in velocity. In a system with an excessive concentration of substrate, the velocity depends on the concentration of ferment alone; within certain limits, variations in the substrate have no result. In a system with an excessive concentration of ferment, the velocity depends on the concentration of substrate alone; within certain limits variations in the concentration of ferment have no result. Where both substrate and ferment are in excessive concentration, irregular and bizarre results will be obtained. In the zone of medium concentrations, the velocity of the transformation is a function of the mass of substrate, and the intensity of the acceleration is a function of the concentration of ferment, but there is an interdependence. The intensity of the fermentative acceleration accomplished by a constant concentration of ferment will vary to some extent with the concentration of the substrate. For different concentrations of substrate the ferment seems to set a different pace, a condition that suggests a sort of a stoichiometric relation. To this variation in intensity of ferment action Henri[1] and Visser attach a high importance, to which reference shall be made later. Most of the experiments with ferments have been conducted within this zone of concentrations. In the last zone of concentration, that of high dilutions, the velocity of the transformation is the function of the substrate concentration, and the intensity of the acceleration is the function of the mass of ferment; and these are independent, just as they should be in a pure catalysis. When operating within this zone, dilution of the entire system has no effect upon the proportionalities of the reaction, while the contrary is observed on dilution of the systems in the other zones of concentration. I believe that if the proper conditions can be attained, this zone will be found in

many fermentations. For the study of fermentation from the kinetic point of view, this zone of concentration is obviously the one to be sought.

It must be insisted upon that the failure of the law of mass action to hold within wide limits of concentrations is not peculiar to the phenomena of fermentations. For many of our best known physico-chemical laws the conformity of fact to theory is confined to narrow limits of conditions. Van't Hoff [3] in the discussion of the general equation for simple reactions, $-\frac{dC_1}{dt} = kC_I\ C_{II}$ . . , remarks that the equation is valid only for quite high dilutions, practically tenth normal. The law of electrolytic dissociation applies only to conditions of infinite dilution, and the variations that are induced by the presence in the system of undissociated molecules are well illustrated in the recent investigation of Jahn. Similar relations were naturally to have been expected in fermentations, and more, for the multiplicity of the conditions should lead us to expect the relations here to be still much more complex.

A further consideration must be emphasized. The total concentration of the substrate must be so low that the entire mass of the solution shall be active in the sense of the mass law. But there is in catalytic accelerations possibly a further extension of the concept "active" necessary. For the catalytic acceleration, only that fraction of the substrate may be considered active which in combination with the catalysor is engaged in the series of intermediary reactions that we believe constitute the modus operandi of these accelerations. This depends obviously on a certain relationship between the concentrations of substrate and ferment. In the zone of high dilution of substrate and moderate dilution of ferment alone may we expect to find the condition that all of the substrate shall be in relation to ferment, and thus active in the full sense; here alone the law can be fulfilled. To what extent this consideration enters into the factor of "ferment intensity" is not known. That the fact holds for pure catalyses may be seen in the acid hydrolysis of starch and protein, where quantitative results may be secured that are very similar to those seen in fermentations. In the acid inversion of disaccharides the disturbances are not noted, but in the acid hydrolysis of the

complex starch the phenomena are as observable as in fermentations.

Armstrong, in his discussion of his results of the study of the inversion of disaccharides, has lucidly stated the conditions that are to be expected with different relations of concentrations. His statements may be quoted in some detail.

"The proportion of the total substrate present combined with the enzyme and undergoing change at any one moment may be regarded as the active mass of the substrate. * * * On the hypothesis that the enzyme combines with the substrate, the active mass of the latter will be that portion of the whole S which is in combination with an amount of enzyme e; it will be convenient to speak of the combination $s + e$ as the active system." He then proceeds to discuss the possible relations. "Case I, in which, whatever the amount of substrate present, the quantity of enzyme is relatively small. Case II, in which there is a difference from Case I, inasmuch as the quantity of enzyme is relatively considerable. Case III, in which the amount of enzyme diminishes as the action proceeds. Case IV, in which the amount of substrate is varied. Case I. As action proceeds, since the magnitude of the active system depends on the amount of the enzyme present, it is obvious that, in the initial stages, if the total amount of substrate S be large compared with s, the enzyme will be in presence of enough substrate molecules to establish the maximum possible number of effective combinations; or in other words, the magnitude of the active system will remain constant and the change will be expressible as a linear function of the time. As the action proceeds further, the amount of S of substrate present decreases until it is no longer negligible compared with that of the active part s, and hence the enzyme will no longer effect the maximum possible number of combinations; the proportion s undergoing change will then be a function of the total mass, and the formation of active systems will be governed by the law of mass action. The rate of change will be a logarithmic function of the time. Case II. If, on the other hand, the quantity of enzyme used be relatively large, the active mass will be a function of the total mass from the very beginning of the experiment, so that the linear part of the change will

escape notice. * * * Stated shortly, the law of mass action is applicable only to the period during which a constant relatively large proportion of enzyme is present together with a continually decreasing amount of substrate, but uninfluenced by the products of change." * * * Case III does not concern us here. "Case IV. When the amount of enzyme and water is kept constant, while that of the substrate is increased, it may be supposed that the magnitude of the active system will increase until $s + e$ reaches a maximum, a definite equilibrium being established between enzyme, substrate and water, the whole of the enzyme perhaps being combined with the substrate. * * * If $s + e$ remains unaltered, whatever the proportion of substrate present beyond a certain minimum, a constant amount of substrate will undergo change in a given time."

Measurement of the reaction. Frequently we are not in a position to measure in an accurate analytic manner the products of the reaction. It is rare that we have the opportunity to measure the products with a poloriscope, by a direct accurate titration or by a determination of conductivity. More often we need to undertake a formal isolation of the desired product and then identify it and measure it by means of some chemical behavior. In some instances such a procedure may yield accurate results, but it may not be feasible to do this in the number of determinations necessary in a measurement of reaction velocity. Sometimes the product may not be accurately estimated in any way, as in the case of the digestion of protein.

A more serious complication arises with a great many fermentations in which the reaction occupies not one but many stages. These mediated catalyses exceed the direct catalyses in the organic world. We have substrate + water = product_1 + product_1; product_1 + water = $p_2 + p_2$; etc., etc., and finally p_n + water = endproduct + endproduct. These really represent a series of successive catalyses. The reactions however do not progress in complete stages; that is, all of the substrate is not first converted into product_1; and all of product_1 then converted into product_2 and so on until the endproducts are reached. On the contrary, several of the products may be found in the mixture in a particular moment. Each of these stages represents

work. Now in a fermentation experiment under such conditions, what shall one measure? Obviously if one wished to measure the reduction in the substrate one must measure product$_1$, which cannot be done because the stage is not completed en bloc. If one measures the endproduct, what one really measures, as Henri has pointed out, is the appearance of the endproduct and not the conversion of the substrate. And yet the measurement of the endproduct may be only possible measurement. Under these circumstances Henri rearranges the equation $C = \frac{1}{t} log. \frac{A}{(A-x)}$ so that under A we understand the quantity of endproduct when the reaction is completed and under x the quantity of endproduct formed in the time t. Under these circumstances it becomes possible to work with fermentations involving reactions in many stages, though in some instances the results are very irregular. It is apparent that while a positive result would indicate that the law holds good for the particular fermentation, a negative result would not indicate the converse; it would indicate simply nothing, beyond that the conditions of experimentation were too uncontrolled to yield results susceptible of interpretation. The majority of the fermentations of great biological importance belong to this class of mediated catalyses; no highly organized body like cellulose, starch, protein, may be hydrolyzed into simple chrystalline endproducts in a single main reaction. It must be realized that this constitutes not an analytical but a fundamental difficulty. The equation $-\frac{dx}{dt} = C\,(A-x)$ presupposes that the substance undergoing transformation exists in each moment in the system either in the form of unaltered substrate A or of product x. The intermediary reactions from the state A to x are not held to occupy measurable time. If now the substrate in the moment of analysis exists in part in the state of e, f, or more states, and not either as A or x, the equation cannot apply. This is precisely what occurs in the digestion of complex substances like protein and starch, and under these circumstances one cannot be surprised that the fermentations of these substances do not follow closely the law of mass action as expressed in the equation.

Reversion of the Reaction.—The equation for the simple monomolecular reaction does not contemplate a reversion of the

reaction. For fermentations, however, we postulate theoretically such a reversion. If this reversion occur with anything like measurable rapidity, the constants obtained with the use of the equation cannot be identical and an entirely different mathematical formulation will be necessary. That a certain degree of reversion occurs in actual fermentations has been practically assumed on the basis of three facts: the substrate is never completely fermented; removal of the products increases the completeness of the fermentation, and reinaugurates it after it has ceased; and the removal of the products increases the rapidity of the fermentation. It must, however, not be overlooked that two of these results could be due to chemical influence of the products upon the ferment. As a matter of fact, the results of the many known experiments in reversions by ferment action (in which the ferment has been mixed with the products of the reaction) has been to indicate that reversions occur with great slowness as compared to the reaction in the other direction. If one attempts to incorporate into the equation of a monomolecular reaction as a conditioning factor that rapidity of reversion experimentally observed in direct tests, the deviation will be almost nil. In addition to this, the station of equilibrium is for most substrates so near to a completed reaction that reversion could scarcely be held to modify to any appreciable extent the mathematical expression of a ferment reaction. Nevertheless, the actual solution of the extent of this variable lies not in mathematical considerations, but in the direct experiment. It may be one of the reasons why the experimental velocities in fermentations do not always agree with the mathematical predications. To the factor of reversion special attention has been called by Visser, and we shall consider his studies in detail later.

Concentration of Ferment. It is as difficult to obtain and maintain a constant concentration of the ferment as of the substrate. The activities of ferments as well as their stabilities seem to depend to a marked degree upon the methods of preparation and conservation. All ferments contain more or less extraneous matter; probably in most instances the percentage of the actual ferment is but a small fraction of the weight of the preparation employed. Under such circumstances it is not

possible to define the exact initial concentration of ferment, and this results in an indeterminate error in the course of series of experiments.

The lack of regularity between the chemical composition and enzymic activity of a ferment is a condition not peculiar to ferments, but is an unfortunate quality of all colloids, for the designation of which van Bemmelen has amplified the use of the term hysterisis. The age, origin, method of preparation and in short every incident in the history of a colloid tend to influence its chemical qualities. Further than this, once prepared and conserved under constant condition, colloids tend slowly to alterations that may in general be described as changes towards crystalloid qualities. This denaturation could be properly conceived to lead to a reduction in the dynamically active mass of the colloid.

Of far greater importance than the difficulty in determining the initial concentration is the impossibility of maintaining the concentration. Ferments as a class have been held to differ from the inorganic catalysors in that the latter emerge from the completed reaction unchanged. This is not strictly true, for the colloid metals are subject to similar reduction in their catalytic properties. In some instances the inactivation is accompanied by precipitation in a granular amorphous form, but in other instances the appearances of the colloid have undergone no change. The inactivation proceeds more rapidly at high temperatures, and seems to affect old suspensions more than fresh preparations.

All ferments are more or less altered during the course of a fermentation. Upon the supposition that we are dealing with pure conditions, there are apparently three conceivable sources for these alterations, namely: reactions with the substrate, the products, or with the solvent. We have almost no knowledge of reactions between the substrate and ferment that would result in inactivation of the ferment. MacIntosh has furnished a good illustration of this relation in the acceleration of the reduction of hydrogen peroxide by colloidal silver. The substrate combines with a portion of the silver to form a compound that is catalytically inactive. We have little knowledge of the inactivation of ferments by reactions with the products. I have

determined that trypsin is more rapidly destroyed in a solution of amido-acids (products of tryptic digestion) than in simple watery solution. Vegetable lipase is somewhat sensitive to acids, and is destroyed more rapidly in even moderate concentrations of acetic acid than in distilled water. In all probability however the chief reaction resulting in the destruction of the ferment is hydrolysis upon the part of the solvent. Just as the albumin or ester constituting the substrate undergoes on suspension in water a slow hydrolytic cleavage of which the digestion is but the acceleration, so ferments on suspension in water undergo hydrolysis; and as the products are not active in the catalytic sense, the active concentration of the ferment is thus steadily reduced during the progress of the experiment. The two hydrolyses, of the substrate and of the ferment, proceed side by side, probably entirely independently. The hydrolysis of the ferment is of course accelerated by increase in temperature, and seems to follow the usual rule for such increase. I have found that the destruction of vegetable lipase proceeds closely according to this rule, while the destruction of trypsin proceeds rather more rapidly. The possible extent of such an inactivation will be understood when one realizes that in some instances a solution of trypsin may be inactivated one-fifth in an hour; and the degree of disturbance that must follow becomes apparent when we consider that some half dozen hours might be required for a digestion experiment with such a solution. In their resistance to hydrolysis ferments however vary widely. The inactivation of the ferment proceeds usually more rapidly in simple solution in water than during the course of a fermentation experiment; this has been shown true for amylase, invertase, trypsin, vegetable lipase, and for zymase. The natural interpretation of this phenomenon is that during the course of the fermentation the ferment is passing through intermediary reactions with the substrate, and that when thus combined its hydrolysis is suspended. The free ferment adds water to undergo cleavage; in the complex substrate-ferment this reaction does not occur.

Tammann, who first studied this matter, attempted to allow for this inactivation by the insertion of a corresponding factor in the formula for reaction velocity. Upon the assumption that

the reaction in each moment is proportional to the momentary concentration of the substrate and of the unaltered ferment, the velocity would be expressed in the following equation: $-\frac{dx}{dt} = C\ (A - x)\ (F - y)$. F is the original mass of ferment, y the ferment inactivated in the time t, the other signs are as before. Tammann measured the destruction of emulsine on heating dry and in solution, and found the reaction in water to be of the first order. The data for the destruction of the ferment he then inserted into the equation above given, following which the constant for the main reaction could be calculated. Tammann made the statement that the constant for the destruction of the ferment is independent of the ferment concentration, and the same for all preparations. This statement is at variance with the truth; the destruction of a ferment by heat varies much with the history of the preparation.

This formula, however, fails, because the velocity of hydrolysis of the ferment is not the same during the course of a fermentation as by itself, but is less. On account of the fact that the ferment when combined with the substrate is resistant to hydrolysis, one must for the purposes of the calculation of the hydrolysis of the ferment, distinguish between the total concentration of the ferment and the active mass, the latter being represented by that portion of the ferment not in combination with the substrate. In general terms therefore, the active mass of the catalysor for the reaction of its own hydrolysis would be inversely to the active mass of the substrate of the main reaction—the hydrolysible catalysor would increase proportionally as the substrate diminishes, and the velocity of the destruction of the ferment would be inversely proportional to the mass of the substrate. As the fermentation progresses, the velocity of the hydrolysis of the ferment will approach the velocity noted in the direct test, because the concentration of the substrate is steadily diminishing, and upon this substrate the repression of the hydrolysis depends. While the cleavage of the substrate is following a logarithmic curve downwards, the hydrolysis of the ferment is following a logarithmic curve upwards. And since upon the basis of the available experimental data this deviation cannot be

experimentally determined, the results of the calculations do not in practice conform to the findings.

Inactivation of the ferment may be produced also by reactions with extraneous substances. Theoretically it ought always to be possible to distinguish between inactivation and destruction of a ferment; practically this may not be always possible. The phenomenon is very frequent in actual experimental work, and may also be provoked in inorganic catalyses. The accelerating action of iron salts may be abolished by the presence of acetic or oxalic acid; the accelerating action of colloidal platinum is depressed by hydrocyanic acid or a thiosulphate as well as by alkalies, etc. These influences are not all of one nature; in some the ferment or catalysor is destroyed; in others the action is inhibited though the substance is not altered, for when the depressing body is removed the original activity of acceleration returns. Colloidal platinum again affords a striking illustration: the inhibition of its catalytic acceleration by hydrocyanic acid, carbon monoxide or phosphorus will pass away with the removal of those substances, while the inhibition following the addition of sulphuretted arsenic, iodine or mercuric chloride remains after the removal of those bodies. For the natural ferments the negatively catalytic influences are exceedingly numerous, and in nearly all instances they effect a permanent inactivation of the ferments. Many of these substances act undoubtedly by accelerating the hydrolytic cleavage of the ferment, that is, they are positive catalysors to the hydrolysis of the ferment. Such is almost certainly the nature of the influence of acids and alkalies.

Stimulation of the ferment by the presence of substances not in themselves accelerators is very frequently observed in connection with fermentations. Thus a trace of acid aids the action of invertase, vegetable lipase, and of the ferments of the pepsin group; a trace of alkali aids many reductions and also the fermentations of the trypsin group. Many salts have similar actions, as have innumerable other substances. These zymo-excitors seem to have two things in common: an optimum concentration and an optimum temperature. A good illustration of these facts (and one that has the further value that it also illustrates the

identity of the conditions in the organic and inorganic worlds) is to be found in the zymo-excitation of alkali upon the reduction of hydrogen peroxide by colloidal platinum and oxydase of animal origin; for both of these reactions one may obtain a curve of the influence of increasing alkali-content with a well defined maximum, and for different alkali-content also a curve of temperature influence with a well defined maximum. Some instances of zymo-depression and zymo-stimulation will be considered in detail in connection with the discussion of particular fermentations.

Station of equilibrium. We have to deal here with a most interesting phase of the question of fermentations. Pure positive catalysors do not bring about any translocation of the station of equilibrium. When such a thing occurs in an inorganic catalysis it is because some one of the possible factors mentioned has intervened. But in the domain of fermentations we encounter a new state of affairs, namely that reactions in themselves practically complete and unlimited, and which remain practically complete reactions when accelerated by inorganic catalysors like acids, seem to become limited reactions when accelerated by ferments. Many ferments seem practically unable to carry through their accelerations to the point of complete conversion of the substrate into the products that is observed when inorganic catalysors are employed. This is not true of all ferments, but of a great many; and the phenomenon is especially observed in the hydrolysis of starch, glucosides and protein. The condition was first described by Tammann when working with amygdaline, although the direct observation of the limited conversion of starch had been made by Payen.

The facts are as follows: A certain reaction when accelerated with hydrogen ions is practically a complete reaction. Let us say the condition of the system may be represented by the relations substrate 1: products 99. At this point the reactions in each direction are equal; the tendency to combination upon the part of the products is very slight since with all the mass of products the combination is only able to balance the cleavage of the substrate when its mass has fallen to 1 per cent. When this same reaction is accelerated by a ferment the cleavage may

not be nearly so complete, and at the close the relations may be expressed something like this: substrate 15: products 85. Does this apparent shifting in the point of equilibrium correspond to a real translocation of the station of equilibrium, to a change in the constant of equilibrium? There are many facts that tend to indicate that such may be really the case. The addition of more substrate to the mixture after the reaction has ceased to progress will serve to reinaugurate the reaction. When the products of the reaction are added early in the course of the experiment, the reaction will cease sooner than otherwise, and cease sooner proportionately to the quantity of products added. On the other hand, when the products are removed from the system, the reaction will be reinaugurated. Concentration or dilution of the volume will also disturb the apparent equilibrium, and an increase of temperature will cause the reaction to recommence in a system that had become quiescent. Finally the addition of further ferment may reinaugurate the reaction, although it can be easily shown that an abundance of active ferment was still present in the mixture. The only possible direct interpretation of the last fact is that the ferment is reacting with the components of the system, probably with the substrate, and possibly in a relation of definite proportions. By repeated additions of ferment it may be possible in some instances to complete the reaction, provided only that the initial concentration of the system was sufficiently diluted. These various facts are identical with those that hold for a translocation of the station of equilibrium in a true reaction of measurable reversibility, except that in these cases the further addition of catalysor will not shift the point of equilibrium. The least difficult interpretation is that the ferment has entered into reactions with the components of the original system, that the station of equilibrium has been shifted thereby and that the reaction to the left, the reversion, is so greatly augmented as to maintain the new equilibrium. As Bredig[2] has succinctly expressed it, the equilibrium is not determined by the substrate and the products, but by the substrate, the products and the ferment. The exact nature of the phenomenon is not at all clear. As a matter of fact, the observation has not been confined to ferments, for as Musculus, Wohl and others

have shown, under certain conditions the acid hydrolysis of starch is not complete, and in fact the reversion of this acid hydrolysis has been made highly probable.

When to a reaction naturally limited and measurably reversible one adds a ferment, how is the equilibrium affected? There has been very little work done upon this class of reactions. Obviously a ferment could by reacting with the components of the system alter the constant of equilibrium as readily as in the case of a reaction that is naturally quite complete. I have studied the question by experimentation upon various esters with a vegetable lipase, and I have never been able to find that the constant of equilibrium has been shifted. Apparently this lipase at least acts as a pure catalysor. The details of these experiments will be furnished later.

CATALYSIS IN HETEROGENEOUS SYSTEMS.

What has been said heretofore applies specifically only to homogeneous systems. Now in many reactions of fermentation, as well as in many organic catalyses, we have to deal with heterogeneous systems, and very important physical deviations are here presented. We have furthermore to deal with different combinations of conditions. The substrate may be solid, the solvent fluid and the catalysor fluid. Or the substrate may be in homogeneous solution, while the catalysor may be solid. There are even conditions in which the products may be heterogeneous, as in the enzymic reaction in which amorphous sulphur is produced from hyposulphite. And lastly we have the condition, common in the world of living matter, of a suspended colloidal catalysor accelerating the reaction of a colloidal substrate suspended in water.

Let us first consider the relations when the substrate is colloidal or solid, the catalysor fluid. When such a body is suspended in water, the same relations will hold that were described by Noyes and Whitney for the conditions of solution of a substance in water. Each particle of the solid is to be conceived as surrounded by an infinitely thin film of saturated solvent, and if the general bulk of the solvent be kept homogeneous by proper stirring, the velocity of solution will be proportional to the dif-

ference between the concentration of a saturated solution and of the particular saturation present in a particular moment, in accordance with the formula $\frac{dx}{dt} = k\ (C - c)$, C being the concentration of saturation and c that concentration actually present in a particular moment. The surface of the solid must be constant. These relations were confirmed by Bruner and Tolloczko and lately by Brunner.

If on the other hand the substrate is fluid or soluble and the catalysor solid, the relations will be, so to speak, reversed. The reaction must be conceived to occur only at the film of contact of the particle with the solution, and the substrate must be brought to this film and the products removed.

Systematic authors like Ostwald have in general applied the law of mass action and the van't Hoff theory of the order of a reaction to heterogeneous systems, the formulae being modified to meet the complicated conditions. Under such circumstances the progress of the reaction is held to take place only in the film of contact between the two phases, and is there proportional to the dimensions of the surface of contact; but otherwise it follows the general law and is proportional to the mass of the reacting body or bodies, it being assumed that the homogeneity of the general bulk of the solvent is maintained. Obviously the conditions would vary, depending upon whether the dimensions of the surface of contact are constant, increase or diminish. Before the work of Bredig this general interpretation had been almost unconsciously adopted also for catalytic reactions, although the facts that the surface of contact in the system is steadily decreasing during the progress of the reaction, and that the products of the reaction are not removed, obviously render the relations very complex. For metallic colloids the suspended particles may be assumed to be symmetrical, probably globular, and under these conditions there is a definite relation between quantity and surface; for ferments, however, the shape of the particles is probably amorphous and of no regular symmetry, so that here the alteration in the dimensions of the surface of contact with the progress in the reaction cannot be even surmised.

Under the conception of the nature of suspensions of the so-called stable colloids first proposed by Quincke and van Bem-

melen and since widely amplified by many investigators, such a colloid suspended in water forms two phases:—a water-poor and a water-rich phase, in short, an aqueous and a colloidal phase. When a third substance is dissolved in such a two-phase system, it is distributed between the two. The substance will be taken up in two ways: partly by adsorption at the film of the colloidal phase, partly in solution in both phases. Now a portion of the substance that has entered into the colloidal phase is irreversibly bound, a larger portion however may be withdrawn. The coefficient of distribution of the substance in the two phases will obviously depend in part upon the concentrations and in part upon the chemical relations that determine for different substances the extent of the irreversible combination. If now the colloid happens to be a ferment, and the dissolved substance the substrate of a fermentation, it is clear that the law of mass action cannot be applied to the reaction under the simple assumption that the velocity of the reaction is proportional to the active mass of the substrate and to the dimensions of the film of contact. In such an experiment, account must be taken of the factor of the coefficient of distribution, and also of the velocity with which this distribution is effected, since with the progress of the reaction, this would be of influence. If both the substrate and the ferment be stable colloids, the situation would be only the more complicated. The difference between the stable colloids, like starch and protein, and the unstable colloids, like the metallic suspensions, must never be overlooked; and they are associated with such pronounced differences in physical behavior that we are not permitted to apply directly to the stable colloids the results of investigations with unstable colloids. While therefore it must be conceded that the law of mass action may not be applied to fermentations in the full theoretical sense, the actual question is: To what extent do the factors of the coefficient and velocity of distribution produce deviations in the operation of the law of mass action? This is a question for experimentation, and there is very little data bearing upon it. It is certain that the relations are different in different fermentations.

Henri[2] has attempted a mathematical characterization of this point of view. The process of a fermentation is divided into

two general parts: the movement of the substrate to the film of colloidal ferment; and the chemical reaction at the film. The movement of the substrate to the particle is again divided into two parts: the relation of the coefficient of distribution, and the velocity of this distribution. The particles of ferment are looked upon as stable colloids; there is in the system an equilibrium between the ferment particles and the inter-particular fluid, depending in part upon the mass of the fluid, in part upon the properties of the ferment particles. The water content of the particles may be increased or decreased by alterations in the mass of water. The particles of ferment effect adsorption of substances in the solution, and this adsorption is in part irreversible, in large part however reversible. If to a liter of a colloidal solution of this sort we add an amount a of a soluble substance, the volume of the colloidal phase v will be notably altered, the volume of the water phase V on the contrary very little. The substance added will be divided between the two phases V and v. The portion that has entered into the colloid phase we will term b; in the water phase there remains therefore $a - b$. The concentration in the water phase therefore equals $\frac{A-b}{V} = C_1$. The concentration in the colloid phase is $\frac{b}{v} C_2$. Since $V + v = 1$, $C_2 = \frac{b}{1-V}$. The portion of the substance held by the colloid b is held partly in reversible, partly in irreversible absorption. We will term the portion held in reversible absorption c, and the portion held irreversibly will be $b - c$. b and c will vary relatively with the nature of the substance, the colloid and also the medium of solution. The concentration of the substance a held in reversible absorption c equals $C_3 = \frac{c}{b} = \frac{c}{1-V}$. A relation of equilibrium must hold between C_1 and C_3. The actual chemical reaction at the film of contact is considered by Henri to be proportional to the mass of the substrate; the mass of the substrate is, however, dependent on this relationship of equilibria. It is obvious that this point of view is much more in harmony with our knowledge of the colloid state than the older idea that the reaction under these circumstances was proportional to the dimensions of the surface

Quite recently Nernst has defined a new point of view in the contemplation of the velocity of reactions in heterogeneous systems. Nernst has amplified the principle of Noyes of the solution velocity to include the apparent velocity of reaction, and to define just what an experimental velocity in a heterogeneous system really means. There are obviously three main processes in such a reaction: the passage of substance through the surface of contact between the two phases, the boundary film; the chemical reaction in one of the two phases; and diffusion to and away from the boundary film. Nernst considers the first process to occur with such rapidity as to have no influence upon the relations. If the chemical reaction occurs with rapidity, as Nernst believes to be the usual condition, it is apparent that the progression of the reaction in time depends only upon the velocity of diffusion. If the reaction be rapid as compared to the diffusion, equilibrium will exist at the surface of contact, and if proper mixing be provided for, the velocity of reaction represents simply the velocity of diffusion of the substrate to the surface of contact. The conception is probably best stated in the words of Nernst:—

"Many facts have led to the assumption that equilibrium is established with extraordinary rapidity at the surface of contact of two phases. Such a condition is indeed a theoretically natural assumption, because at the surface of separation of two phases, as infinitely approximated points, marked differences in chemical potential would appear, and these would obviously produce much chemical energy and lead to great rapidity of reaction. This means nothing more than that in each moment the equilibrium is established very rapidly in the immediate neighborhood of the surface of separation. If one assumes, what is mathematically more probable, that the surface of contact is not a mathematical point but rather a narrow area of transition, we are nevertheless still concerned with dimensions of the order of the sphere of activity of molecular potentials; and though we can then no longer speak of an infinite velocity of reaction, we shall still be dealing with such velocities as are for practical purposes infinite. When we consider a chemical reaction from this point of view, for example the solution of magnesia in dilute

acids, we assume that the magnesia is in each moment in equilibrium with the solution, that is, the solution in immediate proximity to the magnesia is saturated and therefore alkaline. The diffusing acid will be entirely neutralized at the surface of contact; the velocity of solution of the magnesia depends solely upon the velocity with which the acid diffuses to the layer of contact of solvent and magnesia.

"In recent times the van't Hoff theory of the order of a reaction, that is, the deduction of the number of reacting molecules from the progression of a reaction, has been often applied to reactions in heterogeneous systems. When one considers that this theory rests upon the calculation of the probability of the kinetic collision of two or more molecules in the gaseous state or in dilute solution, it becomes clear that there is no sense in its application to heterogeneous systems, or at least that there is for such application no theoretical foundation extant. The above mentioned considerations, however, teach us that the application of the van't Hoff theory to heterogeneous systems is not only without direct foundation, but indeed entirely improper, because in reactions in a heterogeneous system, in so far as the reactions occur only at the surface of contact of the two phases, the velocity is partly or entirely dependent upon the velocity of diffusion, which has no connection with the order of reactions."

(That this reasoning need not hold true in chemical reactions of this type is illustrated by the work of Haber on the electrolytic reduction of nitro-benzene; the reaction at the surface of the electrode was found to be slow compared with the velocity of diffusion to the electrode. As contrasted with the total denial of the application of the kinetic theory of the order of a reaction to a heterogeneous system, it may be pointed out that Senter has suggested that the Brownian movements in the particles of a colloid may in a sense be compared to the molecular movements postulated in a homogeneous solution by the kinetic theory. The general proposition that chemical reactions must occur with infinite velocity in the film of contact of two phases has also been denied on thermodynamic grounds by Sand, who has adduced experimental illustrations to the contrary.)

"A special instance of heterogeneous chemical reactions is afforded by the accelerations of reactions by colloidal catalysors such as platinum-asbestos, Bredig solutions, etc. Since these reactions probably progress solely upon the surface of the catalysor, the velocity will in no way depend upon the mechanism of the particular reaction; if the catalysor maintains its integrity through the course of the reaction (which cannot be foreseen in advance), and carries through its reaction on the surface of contact with practically infinite rapidity, the velocity will depend upon the diffusion of the reacting bodies to the catalysor."

While it is too early to pass judgment upon this theory, whose fundamental import must be apparent, several general considerations may be pointed out. One is that the relations in fermentations will be much more complex, because we have here often a colloidal substrate and a colloidal ferment suspended in water, instead of having a heterogenicity depending upon only one member of the system. Since in the case of a colloidal substrate the reaction is held to occur on the surface of its particles, and in the case of a colloidal catalysor the reaction is held to occur on the surface of its particles, when these conditions are united in one system, the reaction ought to be very slow; experience, however, has taught us that some of these reactions are quite rapid. Secondly, the Nernst reasoning is based upon the assumption that the solid body is so slightly soluble in the medium of the reaction that it does not participate appreciably in the diffusion. This condition will in all probability be found not to hold good for many of the pseudo-colloidal substrates employed in fermentations. Thirdly, the theory assumes that the catalysor is not altered in the course of the reaction, whereas in most fermentations the contrary is the case.

An argument against the Nernst theory lies in the temperature coefficient of fermentations. The velocity of chemical reactions is greatly accelerated by increase in temperature; the velocity of diffusion only slightly. Now in the case of most ferments an increase in temperature is followed by the increase in velocity that would be expected in a chemical reaction—it is usually more than doubled in 10 degrees—far more than would be expected were a process of diffusion alone concerned.

Another objection is that proportional increases in viscosity in the fermenting system, due to the addition of different substances, ought to exert proportional retardations of the velocity, if that velocity express simply the diffusion velocity. This is not the case. Similarly, in heterogeneous reactions of known chemical nature, variations in concentration and viscosity ought to produce the same variations in velocity as in fermentation experiments; but this has not been observed to be true.

Lastly it must be pointed out that the term colloid does not correspond to a fixed quality, but to a tendency to physical qualities that is more or less pronounced in different substances. For Graham the colloid was the non-diffusible body, the crystalloid the diffusible body. Colloids confer upon their solutions or suspensions a very slight depression of the freezing point or elevation of the boiling point, and possess a very low osmotic pressure, all of which indicate that the work necessary to effect the separation of the colloid from the medium is small; with crystalloids the contrary is true. Like all suspensions, colloidal solutions display peculiar conditions of precipitation and coagulation, and are very active in all that relates to surface tension and adsorption. But it is not at all true that all chemical bodies belong to one or the other of these classes. On the contrary, as indicated by Spring and Lobry de Brun, there are innumerable intermediary conditions, corresponding to all conceivable gradations from the typical crystalloid to the typical colloid. Not only this, many substances display attributes quite extreme; thus protamines will not crystallize, but will diffuse well and will transport an electrical current, while some higher proteins will crystallize and yet not diffuse with measurable rapidity; and while some are quite insoluble in the true sense, others are quite soluble. Under these circumstances, dealing with bodies displaying all degrees of gradation from the typical crystalloid to the typical colloid, it is difficult to foresee to what extent the theory of Nernst, based upon the qualities of practically pure heterogenicity in the system, will bear the test of experimental application to fermentations. The colloids with which we are dealing in fermentations are in many respects different from the metallic suspensions to which the theory finds direct application. They

react differently to electrolytes, resist precipitation by them, are indeed in some instances protected from precipitation by them, and they have the power to protect genuine colloids from precipitation by electrolytes. Under ultramicroscopic examination, the stable (or pseudo) colloids are seen to possess much smaller particles than the true colloids; and corresponding to this they display crystalloid tendencies—power of diffusion, osmotic pressure, etc. In fact, some of the proteins have as pronounced crystalloidal properties as many dye-stuffs (which are commonly considered as real crystalloids), and scarcely more measurable colloidal properties. Obviously the theory of Nernst cannot be applied to such bodies with complete theoretical validity. That the conditions upon which this theory is based will often be of great influence in the progressions of reactions must be conceded in advance; it is indeed a priori probable that in many instances the experimental velocity will be the result of both a true reaction velocity and the diffusion velocity; but whether it will afford in itself an adequate explanation of all the phenomena is very much to be doubted so far as biological fermentations are concerned. As will be illustrated later, the results in many instances correspond very closely with the requirements of the older law. The investigations of Heimbrodt indicate that with constant conditions of diffusion, the differential equation demanded by the Nernst theory is identical with that for a monomolecular reaction. In other instances, however, such conformity has not been obtained, and it may be in precisely these cases that the conditions elucidated by Nernst have played predominating roles. The cleavage of an insoluble fat by lipase represents, as will be later noted in detail, a probable illustration of a diffusion velocity.

Another factor which must modify the relations in the complex fermentations of biological order is the relations of colloids to each other. The adsorption of stable colloids by one another is not a function of surface tension; it is in part at least a function of composition. The adsorption of a particular colloid by two different colloids even of the same surface tension is not the same. It is the usual teaching that colloids cannot diffuse, but the phenomena of adsorption throw grave doubt upon this state-

ment. Colloidal hydrosols will penetrate hydrogels in a progressive linear manner. That this may not be a true diffusion, but rather a co-solution, a relation of the coefficient of distribution, possibly even a chemical combination with the formation of a new colloidal complex, is freely granted. But the fact will no less indicate that the phenomenon under whatever name it pass must constitute a variable in the reactions in such a system.

In the concrete application of the Nernst theory to fermentations, as proposed by Herzog, it is assumed that the diffusion velocity is the function of the viscosity of the medium, and the equation is erected on magnitudes derived from this consideration. The work of Herzog will be discussed in detail under inversion.

It is necessary further to consider the nature of the relations between the substrate and the ferment. We know on the one hand that colloids enter into complex chemical combinations with other substances. We have evidence that complex combinations of this sort occur in organisms: such are the sugar-protein, the protein-fat, the purin-nucleine, the lecethin-protein complexes. These seem destined to play an important rôle in the chemical physiology of the future. As chemical combinations these complexes are subject to the laws of mass action, equilibrium and partition. On the other hand, we know that colloids form with other substances physical equilibria that van Bemmelen has termed adsorption compounds. Recent investigations seem to indicate that for the typical unstable colloids (metallic hydrosols, silicate, etc.) the laws of mass action, equilibrium and partition do not seem to hold. For the atypical colloids (protein, starch, etc.) these laws seem to tend to hold. It is fairly certain that for some reactions (*e.g.,* the system dye-cellulose) the early occurrence of secondary reactions affecting the one component (the dye) are responsible for the non-fulfillment of these laws and the cause of the irreversibility of the reaction; such a phenomenon, however, does not constitute a fundamental exception to the laws. Concerning these matters we possess as yet so little quantitative data that definite conclusions are not warranted. Nevertheless, unless we can conceive that when two substances meet in a thin film on the surface of a third indifferent sub-

stance they react with infinite velocity, we shall be compelled to consider the combination of ferment-substrate to be of the nature of a chemical complex rather than of a physical adsorption compound.

In its broadest application, the fact upon which the theory of Nernst is founded, that reactions occur with great velocity at the boundary of contact of two phases, must appear to every one as a fact of the deepest biological significance. When we consider that the cells of the body in their relations to the circulating fluids, indeed the fibrillar and granular parts of cells in their relations to the intercellular fluids, represent precisely just such two-phase systems, we realize the magnitude of the factor with which we are dealing. From the physical point of view, the cellular constructions are simply colloidal phases, hydrogels, the body fluids watery phases, hydrosols. The magnitude of the dimensions of the boundaries of contact of the two phases in a human body is almost inconceivable; and the influence upon the velocity of any reaction under these circumstances as contrasted with the velocity of the same reaction in a simple system of the same bulk must be enormous. Though it is a pure speculation, I cannot refrain from expressing the thought that to this factor may be ascribed, in part at least, the great difference in velocity everywhere to be observed between reactions in vivo and in vitro.

Very recently (in fact, since these lectures were delivered) Henri[3] has advanced some general ideas of ferment action which combine the chemical point of view of the reaction velocity with the physical properties of a colloidal system. His views may be summarized as follows: Soluble ferments are solutions of stable colloids. Colloids are heterogeneous media formed of granules suspended in an intergranicular fluid. Stable colloids are formed of granules rich in water. Between the granules and the intergranicular fluid a state of equilibrium exists; the composition of the granules has a direct relation to that of the liquid. In the variation of the composition of the granules one must distinguish two factors: variation in the water content, and variation in the adsorption, by the granules, of substance dissolved in the intergranicular fluid. Substance adsorbed by the granules is in two forms: that adsorbed irreversibly, and that held in reversible

combination. The division of the solute between the granules and the intergranicular fluid follows a law of coefficient of distribution. The essential character of the action of ferments lies in a relation between the reaction velocity and the concentration of the solution; this relation, however, depends upon the coefficient of distribution of the substance between the granules and the intergranicular fluid.

Reactions in a heterogeneous system may be divided into two groups: hetereogeneous catalysis, and reactions between substances in two phases. In each of these the reaction may be conceived to occur on the surface of separation of the two phases or in the interior of one of the phases. The velocity of reaction in a heterogeneous system will depend upon the following factors: (*a*) The velocity of the chemical reaction *per se,* which shall be termed *R*. (*b*) The velocity with which the bodies that react arrive at the boundary of contact of the two phases, or penetrate into the interior of the phase where the reaction occurs; this velocity is determined directly to be the coefficient of diffusion of the reacting body *D*. (*c*) The concentration of the reacting bodies at the site of reaction; if the reaction occur in the interior of one of the phases, this will be expressed in the coefficient of distribution. (*d*) The dimensions of the surface of contact of the two phases *S*, and the volume of the phase in which the reaction occurs *V*. I. If *R* be very rapid, the reaction will occur solely at the boundary of contact of the two phases, the law of distribution is eliminated, the film of separation of the two phases has a constant thickness and composition, the velocity of diffusion alone is the operative factor. (This is the hypothesis of Nernst.) II. If *R* be relatively slow, *D* and the distribution very rapid, the reaction will occur in the interior of one of the phases, and the reaction velocity will depend on the concentration of the reacting bodies, if *S* and *V* are constant. III. If *R* is rapid, and *S* is variable, the velocity of the reaction occurring at the film of contact of the two phases will depend on the diffusion and the dimensions of the surface *S*. Henri considers that in those fermentations in which the substrate is a crystalloid and the ferment a colloid, the phenomena are to be ranged under II or III. In the case of those fermenta-

tions in which both the substrate and the ferment are colloids, the relations will be very much complex, but they may also be ranged under II or III. Obviously the possible combinations of these several factors are not exhausted by the I, II, and III of Henri. On the assumption that the actual reaction is proportional to the masses of the reacting bodies and is further a function of the partition and equilibrium between the solution and the colloid phase, Henri has developed a general equation theoretically applicable to all fermentations. Henri has as yet published no experimental data bearing directly on the classification of particular fermentations under these headings. That the scheme includes all the possible factors involved in this phenomenon may be granted. To what extent these several factors will be found to be operative in their relations to each other in particular fermentations cannot be foreseen. That difficulties will appear in the formulation of the problem and especially in the different measurements is equally obvious. Nevertheless the scheme is very lucid, and attractive in that it conserves the chemical nature of the phenomenon and locates the adventitious variables that lie outside of it in the domain of the physical properties of colloids, as defined in one of the most current and best elucidated theories of the colloid state. The weakness of the scheme lies in the disregard of the fact that many of the so-called colloids with which we are dealing are more or less atypical, and deviate greatly from the typical colloid in the direction of crystalloidal properties. It has become the fashion in biological circles to attribute the most numerous and diverse of unexplained phenomena to the physical properties of the colloidal state. Valuable as the physical point of view unquestionably is, in the investigation of the concrete problem such general assumptions must be replaced by experimental work.

LITERATURE.

Abelous & Gérard. C. r. Acad. Sc., *129*-50, *164*, 1023.
Armstrong. Proc. Roy. Soc., *74*-511.
Baeyer. Berichte, *3*-68.
Bertrand.[1] C. r. Acad. Sc., *118*-1215; *120*-266; *121*-166, 783; *122*-900, 1132, 1215; *123*-463.
Bertrand.[2] C. r. Acad. Sc., *126*-702, 842, 984; *127*-124, 728.
Bredig.[1] Die Anorgan. Fermente.
Bredig.[2] Ergebnisse der Physiologie, I-191.
Brode. Zeitschr. f. physik. Chem., *37*-257.
Bruner & Tolloczko. Zeitschr. f. physik. Chem., *35*-283.
Zeitschr. f. anorgan. Chem., *28*-314; *35*-23.
Brunner. Zeitschr. f. physik. Chem., *47*-56.
Buriau. Zeitschr. f. physiol. Chem., *43*-497.
Engler & Weissberg. Krit. Stud. u. d. Vorg. d. Autoxydation.
Ernst. Zeitschr. f. physik. Chem., *37*-448.
Erlenmeyer. Berichte, *10*-634.
Euler.[1] Berichte, *37*-3411.
Euler.[2] Berichte, *38*-2551.
Fawsitt. Zeitschr. f. physik. Chem., *41*-601.
Goldschmidt & Reinders. Berichte, *29*-1369, 1899.
Gosio. Atti r. Acad. d. Len., *13*-422, 642.
Rev. d'Igiene, 1892, 201.
Heimbrodt. Ann. d. Physik. (IV), *13*-1028.
Henri.[1] Lois générales de l'Action des Diastases, 85.
Henri.[2] C. r. Soc. Biol., *58*-612.
Zeitschr. f. physik. Chem., *51*-19.
Henri.[3] Zeitschr. f. Electrochem., *11*, 790.
Hoppe-Seyler.[1] Arch. f. d. ges. Physiol., *12*-11.
Hoppe-Seyler.[2] Zeitschr. f. physiol. Chem., *2*-13.
Jacquet. Arch. f. exper. Path. u. Phar., *29*-386.
Jahn. Zeitschr. f. physik. Chem., *50*-129.
Jones. Zeitschr. f. physiol. Chem., *42*-343; *44*-1.
Kastle & Loewenhart. Am. Chem. Jour., *29*-397, 563.
Knietsch. Berichte, *34*-4069.
Knoblauch. Zeitschr. f. physik. Chem., *22*-268.
Koelichen Zeitschr. f. physik. Chem., *33*-129.
Kossel. Zeitschr. f. physiol. Chem., *41*-321; *42*-181.
Kuester. Zeitschr. f. physik. Chem., *18*-161.
Lobry de Bruyn. Rev. Trav. chim. d. Pays-Bas., *19*-236, 251; *23*-155.
Loeb.[1] Berichte, *37*-3593.
Loeb.[2] Zeitschr. f. Electrochem., *11*, 745.
Luther & Weigert. Zeitschr. f. physik. Chem., *51*-297.
MacIntosch. Jour. Physic. Chem., *6*-15.
Nernst. Zeitschr. f. physik. Chem., *47*-52.

Noyes. Zeitschr. f. physik. Chem., *19*-599.
Noyes & Whitney. Zeitschr. f. physik. Chem., *23*-689.
Pawlow. Die Arbeit der Verdauungsdruesen, 33.
Sand. Proc. Ray. Soc., *74*-356.
Zeitschr. f. physik. Chem., *51*, 641.
Schmiedeberg. Arch. f. exper. Path. u. Phar., *14*-379.
Spitzer. Arch. f. d. ges. Physiol., *76*-192.
Spring. Rev. Trav. chim. d. Pays-Bas., *18*-233; *19*-204.
Bull. Acad. Roy. Belg., *37*-174, 300.
Senter. Zeitschr. f. physik. Chem., *51*-698; *52*-737.
Jour. physic. Chem., *9*-311.
Tammann. Zeitschr. f. physik. Chem., *3*-25; *18*-426.
Zeitschr. f. physiol. Chem., *16*-271.
Titoff. Zeitschr. f. physik. Chem., *45*-641.
Turbaba. Zeitschr. f. physik. Chem., *38*-505.
Zeitschr. f. Electrochem., *8*-70.
Van't Hoff.[1] Vorlesungen u. theoret. u. physik. Chem., I, 206.
Van't Hoff.[2] Étud. d. Dynam. chim., 14.
Van't Hoff.[3] Vorlesungen u. theoret. u. physik. Chem., I, 191.
Visser. Reactie-snelheid ect., op Enzymwerkingen. Dissertation, 1904.
Zeitschr. f. physik. Chem., *52*-257.
Wegscheider. Zeitschr. f. physik. Chem., *30*-593; *34*-290.
Wiener. Arch. f. exper. Path. u. Phar., *42*-374.
Wys. Zeitschr. f. physik. Chem., *11*-492; *12*-514.
Young. Jour. Amer. chem. Soc., *24*-297.

FERMENTATION OF CARBOHYDRATES.

In the fermentation of carbohydrates we have to deal with three general groups of ferments. The ferments of one group accelerate the hydrolysis of the polysaccharides to di- or mono-saccharides. Starches are as a rule hydrolyzed only to di-saccharides, which then fall into the second division. There is, on the contrary, some evidence tending to indicate that cellulose may be hydrolyzed directly to mono-saccharides, though it has not been demonstrated that but one ferment is concerned. The ferments of the second group accelerate the inversion of disaccharides to mono-saccharides. These ferments often accompany those of the ferments of the first group, so that their actions are superimposed. The ferments of the third group accelerate the conversion of mono-saccharides into substances like alcohol, acids, etc.

Strictly speaking, as Fischer has insisted, the glucosides should be classed with the di-saccharides rather than with the starches; they consist of combinations of primary sugars with organic acids, alcohols, aldehydes, or aromatic substances; and though they often contain more than one molecule of sugar, it is usually the same sugar, and the two molecules are apparently combined with the other component rather than with each other. Their ferments furthermore are of the type of invertase. It has been observed that other ferments coexist with them, and the results were superimposed, and this led to a great deal of confusion in the early literature.

We shall limit ourselves to the consideration of the relations of six of these ferments: amylase (fermentation of starch), invertase (fermentation of cane sugar), maltase (fermentation of malt sugar), laccase (fermentation of milk sugar), emulsin (fermentation of glucosides), and zymase (fermentation of glucose). Not only are these the most prominent members of their series, but they have been studied in the most thorough manner.

FERMENTATION OF STARCH.

The fermentation of starch shares with alcoholic fermentation the greatest age of investigation. Kirchoff, who discovered the acid hydrolysis of starch, first described the conversion of starch into sugar through the agency of fresh gluten. Dubrunfaut showed that a particular substance in the grain possessed this activity, was present to greatest degree during germination, was soluble in water, and that the sugar produced was not identical with that obtained through the action of acids upon starch. Payen separated the raw ferment from its solutions by precipitation with alcohol. Since that time the action of amylase has been studied to an enormous extent, in large part by the technical chemists engaged in the fabrication of alcohol and maltose, and in the brewing of beer. The presence of amylytic ferment in the saliva was discovered by Leuchs, in the pancreatic secretion by Bouchardat and Soudras, and in the succus entreicus by Roehrmann. It is present also in the liver, in muscular tissue, and in the urine. Whether the traces that may be found in the other organs and tissues of the body represent local formation or simply the deposition from the blood is not known. The presence of amylase in the blood is probably a secondary condition, dependent upon the liver.

Amylase is very widely distributed. In addition to being found in all higher species, it is known to be present in insects, arthropodae, sponges, shell fish, coelenterae, echinoderms, and even in protozoa. It has been described in the eggs of some crustaceans. In all animals there are apparently two locations of the ferment: one in the secretions of the digestive glands, and the other in the liver. In the vegetable kingdom its distribution is equally wide. It is apparently present in seeds during the period of germination, and in many grains to some extent during the resting period. It is also present in trunks, stalks, bulbs, in sprouts of all kinds, and in leaves of plants of all degrees of dignity. It is also present in many fungi, such as different varieties of aspergilli, sacchromyces, in all the common yeasts, in the bacteria that ferment wood, and in many bacteria and cocci,

especially in the cholera vibrio, in the group bacterium termo, the proteus vulgaris, and the bacillus mesentericus vulgatus. The amylytic activity is, however, not a prominent faculty of disease-producing germs in general, though it seems to be a common faculty.

Whether in all these situations we are dealing with the same ferment can only be conjectured. There is on the one hand no reason why the acceleration of the hydrolysis of starch should be limited to one substance, and it is not natural to suppose that all these different plants and animals should be so united upon this one point when they vary so widely in other functions. And, furthermore, the diastatic ferments differ so widely in their relations to different temperatures and to the action of various chemical substances that this affords concrete grounds for attempts at differentiation. At the same time, recent studies in colloids have indicated that different preparations of a colloidal metal of chemical purity will exhibit widely different properties, depending upon the circumstances of preparation, conservation, etc., so that from such differences alone separate chemical entities cannot be inferred. Kjeldahl has shown that when a particular preparation of amylase is exposed for some time to a higher temperature, it will exhibit differences in behavior of surprising extent as compared with the qualities of the parent stock, and in many instances these artificial differences are greater than those observed in different preparations and relied upon to indicate a multiplicity of ferments. As a matter of actual fact, we possess so little definite knowledge upon the matter, knowledge based upon study of pure preparations, that a definite opinion is not warranted.

The same considerations hold true with reference to the individuality of any particular amylase. The reaction consists of two separate functions, the liquefaction and the saccharification of starch. The liquefaction of starch means the conversion of a hydrogel into a hydrosol, and it is difficult to consider this related to the hydrolysis of the starch. Not only are the procedures different, but the relations of temperature and of extraneous substances in the solvent are different for the two procedures. According to the interpretation of Maquenne and Roux,[1] starch

consists of two substances, amylo-cellulose and amylo-pectin. The liquefaction ferment acts on the amylo-pectin, the saccharification ferment acts on the amylo-cellulose. Fernbach and Wolff[1] have described preparations of amylase that had no power of liquefaction, but were active in saccharification. It has been natural therefore to assume that amylase contains two different ferments. If we judge from the history of ferments, this will probably prove to be true, but it has not been demonstrated directly. Furthermore, it has been long claimed that the hydrolytic acceleration is due to two rather than to one ferment; the one being supposed to accelerate the conversion of starch into dextrines, the second to accelerate the conversion of the dextrines into maltose. In the present state of our knowledge, this hypothesis can be neither affirmed nor denied. One must, however, be very careful in grasping at the assumption of a different ferment for each sub-stage of a reaction, otherwise we shall be soon possessed of as many hypothetical ferments as we are supposed to be possessed of anti-bodies.

Wherever found, amylase is apparently a secretion product of cells. It is secreted in an inactive form, as a zymogen, which on suspension in water becomes converted into the active ferment. The quantitative relations of this activation, as well as the fact that it may be accelerated by many substances, leads to the inference that the process of activation is in reality an act of hydrolysis, thus: amylase-zymogen + water = amylase. Some botanists have attempted to separate two forms of amylase, the secretion and the translocation ferment; a chemical differentiation has not been demonstrated. The sum total of our knowledge of the relations in plants suggests that not only does amylase accelerate the formation of maltose from starch, it also accelerates the formation of starch from maltose.

The Hydrolysis of Starch. This has been a bone of great controversy. That the end product is maltose is undoubted. The reaction runs:

Starch + water = maltose + maltose.

$$n\ (C_{12}H_{20}O_{10})_n + n\ H_2O = (C_{12}H_{22}O_{11})_n + (C_{12}H_{22}O_{11})_n.$$

The reaction under favorable conditions may be complete in the sense that the starch has disappeared, but it is incomplete

in the sense that the substance has not all been converted into maltose. Lea has shown that if the products of the reaction be removed from the system, the reaction will be completed. The reaction has many sub-stages; it is a mediated catalysis. In the acid catalysis of starch, similar intermediary stages appear, with the formation of successive dextrinous bodies. The reactions of these dextrines are very indefinite, and one cannot read the work of Musculus, Lintner & Duell, Brown and Morris, Bourquelot, Duclaux, and others without becoming convinced firstly that the number of sub-stages in the reaction is not known, and secondly that for those intermediary stages that seem quite definite, the corresponding products offer at present no definite distinguishing properties. It is difficult to secure an inversion of starch without the persistence of some of these dextrinous substances.

Concerning the actual steps in the hydrolytic cleavage of starch, there are divergent opinions. Here, as elsewhere, the less definite the experimental data, the more uncontrolled the inductions. Based upon the fact that the cleavage is never complete, dextrine always remaining, is the hypothesis that the reaction might be a direct cleavage:

$$\text{Starch} + \text{water} = \text{dextrine} + \text{maltose}.$$

This hypothesis is not in accord with the investigations in fractional fermentation, and the fact of dextrine remaining with the end products is more easily interpreted on the basis of a limited reaction.

A second hypothesis is that successive dextrines are formed, and from the final dextrine maltose is formed.

$$\text{Starch} + \text{water} = \text{dextrine}_1.$$
$$\text{Dextrine}_1 + \text{water} = \text{dextrine}_2.$$
$$\text{Dextrine}_n + \text{water} = \text{maltose} + \text{maltose}.$$

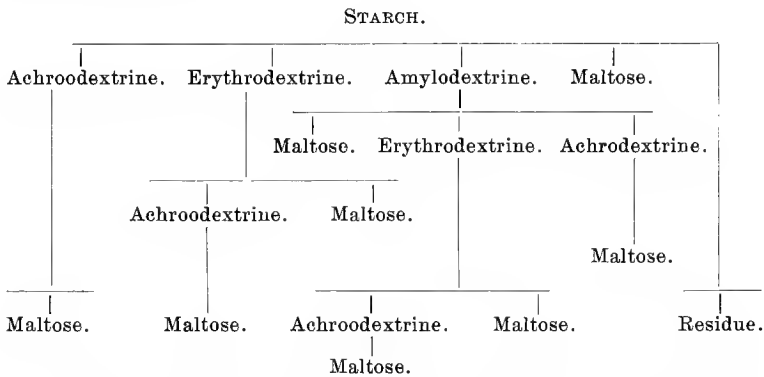
The third hypothesis is that molecules of maltose are successively split of the successive dextrines.

$$\text{Starch} + \text{water} = \text{dextrine}_1 + \text{maltose}.$$
$$\text{Dextrine}_1 + \text{water} = \text{dextrine}_2 + \text{maltose}.$$
$$\text{Dextrine}_n + \text{water} = \text{maltose} + \text{maltose}.$$

As between these two hypotheses, the available data yield no decision. From the point of view of the investigation of the

dynamics of the process, neither would be preferable over the other, since in both we have a series of reactions of which we can measure only the disappearance of the starch and the appearance of the maltose. No method of measuring the conversion of starch into the first dextrine has been established; and the measurement of the maltose is rendered uncertain until we learn that the lower dextrines do not reduce Fehling's solution or disturb its regular reduction by maltose.

According to the latest investigations of Moreau, the reactions comprise a combination of the last two schemes. He gives the following scheme:



Whether oxygen is necessary to the action of amylase has been often discussed. Recent experiments of Sacharow would seem to indicate that it is necessary.

The reaction of the cleavage of glycogen is, so far as known, very similar to that of starch. The intermediary dextrines seem on the basis of color reactions less numerous than in the case of starch, and the reaction usually proceeds with greater rapidity. When a pure amylase is employed, the product is maltose. That glucose is found when mammalian amylase is used is due to the fact that an admixture of maltase is always present. Philoche[1] states that with the use of pancreatic amylase the digestion of glycogen is very like that of soluble starch. With the use of vegetable amylase, on the contrary, the glycogen is much more resistant. The curve of reaction rose rapidly, soon to become almost stationary. Experiments at this stage indicated that the

ferment was unaltered, but that the unconverted glycogen no longer gave the iodine reaction, although it was still perceptible by alcohol.

For the investigation of an amylase a soluble starch should be used. There are several reasons why a raw starch is not suitable. The reactions of liquefaction and saccharification are superimposed, and as they are fundamentally different things, the essential conditions of a fermentation experiment are not fulfilled. The rapidity of the action of an acid or a ferment is dependent to a large extent upon the physical state of the starch: its fineness of subdivision, its coating with cellulose, the lipoids, its dessication. A starch solution should be weak, from one-fourth to one-half per cent. It must be tested for its sugar content. If this be large, it must be removed by dyalization, not a rapid procedure with maltose; if it be low, it may be neglected. The solution should be opalescent; it must not contain floculi; it should not precipitate on standing; it should, in short, be a stable hydrosol. Since there seem to be some differences in the fermentability, *i.e.*, in the chemical resistance of starches from different plants, all the material in a series of experiments should be prepared from one source, and preferably at one operation. For studies of amylase of vegetable origin, glycogen is sometimes preferable to starch, in that it is very soluble in water and seems to present fewer sub-stages in the process of cleavage. In the use of glycogen, however, the absence of maltase from the amylase under investigation must be determined. For investigations with mammalian amylase glycogen cannot be used, because all animal extracts and secretions containing amylase contain also maltase, and the fermentation of the maltose could not be measured. Against the availability of glycogen under any circumstances are the difficulties of preparation in quantity, and its tendency to denaturation during the manipulations attending its isolation and purification.

The Mett method, the use of capillary tubes filled with starch gel, is entirely unadapted to studies on the cleavage of starch, since only the liquefaction of starch is measured with this method. Even this cannot in my experience be measured with regular and satisfactory results.

The measurement of the progress of the hydrolysis of starch is an unsatisfactory operation. The use of color reactions, as those with iodine, is wholly unadapted to accurate work; one cannot in this manner even determine precisely the moment of disappearance of the starch, and the successive stages are still less satisfactorily demarcated. The only feasible measurement is that of the maltose. This cannot be determined by polariscopy, though the reasons for this have not been clearly worked out. The maltose must be determined by chemical methods, best by reduction of copper in Fehlings solution. It must at the outset be realized that the technique of the estimation of maltose by Fehling's solution has not been developed as in the case of glucose, which, thanks to Pflueger, can now be estimated with great accuracy. A preliminary estimation must be always made to determine whether the substrate solution possess any reducing power, since it is a fundamental assumption in the equation that when $t = O$, x is likewise O. In the calculation of the findings the equation must be, so to speak, reversed, as suggested by Henri: A stands for the total maltose at the end of the reaction, x for the maltose formed in the time t. That this use of the equation is not theoretically entirely justified is seen in the fact that the reaction is not complete, but the uncompleted portion is present in the system in the form of a sub-stage product, a dextrine, and not in the form of the substrate starch. Nevertheless the results seem fairly satisfactory, in that they conform to the theoretical expectations.

Kinetics of the Fermentation. The progression of the reaction in the conversion of starch into sugar follows in general terms the regular law. Brown and Glendenning,[1] employing an amylase from malt, obtained figures that were a little too rapid to be accurately represented in a logarythmal curve, but which gave fairly good concordance for the constants when calculated with the empirical equation of Henri: $C = 1 \log \frac{A+x}{A-x}$. Henri,[1] on the contrary, working with ferments from malt and from the pancreas, obtained results closely in accord with the logarythmal curve. In both instances these results were obtained only with dilute solutions; but for the ferment from the malt the conclusions were valid only for solutions below 0.75 per cent., while

with the pancreatic ferment solutions could be employed up to 2 per cent. The acceleration bore a relation proportional to the quantity of ferment.

I have tested the diastatic ferment of saliva, using the fresh human secretion. Comparative researches are difficult because constancy in the ferment cannot be maintained through a prolonged secretion. The substrates were one-quarter, one-half, and three-quarter per cent. solutions of soluble starch, mixed with one-tenth vol. of fresh saliva obtained by the mastication of paraffine. The saliva was distinctly alkaline; no other alkali was added.

Fairly satisfactory experimentation may be done with the salivary amylase if a few precautions are followed. The saliva must be obtained from glands that have had several hours' rest. The mouth should be several times washed with an antiseptic solution, as salicylic acid or benzoyl-acetyl-peroxide. The secretion of saliva should be stimulated by the mastication of pure paraffine. The first portions of the saliva should be rejected; the mucus content is high, the bacterial count high, the ferment concentration low. The filtered saliva should be added to the starch solution at about 30–35°. At this temperature saccharification is less rapid than at 45°; inactivation of the ferment is also less active. For tests of moderate length, the addition of an antiseptic is not necessary. At stated intervals portions are removed, rapidly heated to boiling, and then added to Fehling's solution in a water bath (according to Pflueger), and the reduction of copper measured gravimetrically. It is not advisable to carry the experiment beyond the saccharification of about half the substrate, since the maltase (always present) will more and more appreciably invert the disaccharide into glucose. It is a curious fact that the maltase of saliva will convert maltose into glucose during the course of such an experiment much more rapidly in the case of glycogen than in the case of starch. For this reason the results with starch are quite satisfactory, those with glycogen are quite irregular. The active concentration of amylase in saliva is so different in different times of secretion that comparisons cannot be made except between tests done with the

same saliva at different concentrations of substrate or ferment.

The results of one of such series were as follows:

Substrate ¼%. Temperature 35°.								
t (min.)	30	45	60	75	90	120	150	180
C (x 10⁻⁶)	490	465	455	470	465	455	560	455
Substrate ½%.								
t (min.)	30	45	60	75	90	120	150	180
C (x 10⁻⁶)	430	420	390	415	405	395	430	410
Substrate ¾%.								
t (min.)	30	45	60	75	90	120	150	180
C (x 10⁻⁶)	390	370	385	390	480	370	365	370

The constants were calculated according to the equation $C = \frac{1}{t} \log \frac{A}{A-x}$ used in the reversed manner suggested by Henri: A is the total product when the reaction is complete, and x the amount of product formed in the time t.

The figures cannot be compared with Henri's[2] figures for pancreatic juice because there is no way to allow for the differences in the conditions of experimentation. His constants for a 0.75 per cent. solution at 18°. were:

t (min.)	34	55	94	133	273
C (x 10⁻⁵)	238	222	227	212	200

The constants are quite concordant in each series, but they are not in agreement in the several series. We here encounter a concrete illustration of an experimental finding that is frequently obtained in quantitative studies in fermentations, and which has not been sufficiently appreciated. While we may obtain in the calculations of a series of measurements in a particular experiment constants that exhibit a satisfactory uniformity, this uniformity is not to be obtained in several series carried out with the same ferment concentration but with a different substrate concentration. Theoretically, all concentrations of the substrate (within certain limits) should yield with the same concentration of ferment uniform figures for the velocity constants. The figures given above for amylase illustrate the failure to do this. For this failure to realize the theory many possible explanations may be suggested, but of these, three only are of fundamental import. It is in the first place possible that the intensity of the enzyme is a function not only of its own concentration, but also

of the substrate concentration. To this factor of intensity of ferment action Visser has recently given special attention, but I do not think he has been able to furnish an actual elucidation of the relationship. That the non-uniformity in the constants in different series of substrate concentrations cannot be reduced to a variation in the intensity of the ferment action is made highly probable by the fact that the acceleration of the reaction in different series with the same substrate concentration is proportional to the concentration of ferment. A concrete illustration will make this clear. In three series of tests with respectively one-quarter, one-half, and three-quarter per cent. solutions of soluble starch, and with a uniform ferment concentration, the constants will be fairly uniform in each series, but not at all uniform in the several series. For each of these substrate concentrations, on the other hand, doubling the ferment will double the acceleration. These facts are not in harmony with the hypothesis that the lack of uniformity in the constants in series of only slightly different substrate concentration is due to variations in the intensity of ferment action.

The second explanation would rest the variation in constants on the factor of the coefficient of distribution in the relations of ferment and substrate in the system. That variations in the concentration of the substrate might lead directly to variations in this coefficient, and also in the velocity of the distribution, is apparent. That variations in the mass of ferment would likewise lead to alterations in the coefficient is of course true, but the mass of ferment in an experiment is so small in contrast to the mass of the substrate that we would not expect any deviations due thereto to be apparent in the measurements. At first sight, it might be expected that this factor should be tested, on the assumption that variations in the constants ought to be proportional to variations in the substrate concentration. This could not, however, be expected, unless the coefficient of distribution and the velocity of distribution were functions of the viscosity, which they are not; they are in large part functions of the chemical qualities of the reacting bodies, and these qualities for the substances under consideration cannot be determined. While therefore the extent of this factor cannot be even guessed at, it is

certain that variations in the substrate concentration might be expected to lead to variations in the velocity constants as the result of the operation of the factors o fthe coefficient and velocity of distribution.

The third reason for non-uniformity in the constants lies in the fact that we are dealing with a reaction in many stages. On the assumption that the enzymic acceleration consists of a series of intermediary reactions in each stage (and we are able to measure only the last stage), a uniformity of progression could not be expected with a fixed concentration of catalysor in series of different concentrations of substrate. Experience with reactions in many stages has in many lines of chemical investigation led to uninterpretable results, so that we do not here face a difficulty peculiar to fermentations. That this factor may be of determining importance is suggested by the fact that a similar lack of uniformity in the constants for different substrate concentrations is observed in the hydrolysis of starch with acids.

From the figures it appears that the constants diminish as the substrate concentration increases. The averages for the three series are: one-quarter per cent., 494; one-half per cent., 415; and three-quarters per cent., 390. Between the reductions in the constants and the increases in the substrate concentrations, however, no mathematical relations exist.

In the above considerations, it has been assumed that the ferment was not inactivated or destroyed in the experiment. This condition may be approximately attained with amylase. If the ferment, however, were less stable, further variations in the constants would be expected, since in general terms the hydrolysis of the ferment (its destruction) is proportional to the active mass of the ferment, and this is in general terms inversely proportional to the untransformed substrate; the inactivation of the ferment would therefore proceed differently with different concentrations of substrate.

In all the work on starch it has been found that with solutions of high concentration the transformation in the unit of time did not correspond to the mass of substrate; it was, in fact, usually constant in time. This fact has been used against the idea that the fermentation of these bodies bore a close resem-

blance to the acid hydrolysis of these bodies. As a matter of fact, the results do not support the objection at all. All along the line of work in catalysis it has been recognized that high concentrations of the substrate disturbed the relations. In some of the published work the concentrations have been enormous: Duclaux used solutions from 10 to 40 per cent., Brown from 5 to 20 per cent.! The higher concentrations given amount to veritable pastes. Now one must distinguish between the total mass of the substrate and the active mass. One cannot resist the comparison with the active mass and the total concentration of an acid. The acid acting as a catalysor is active only in proportion to its dissociation; and certainly there is some such distinction in organic molecules, although we may possess as yet no measurable notion of the physical or chemical state to which this activity belongs. It must not be imagined that the law of Wilhelmy holds for the highest concentrations of sugar, not at all. If one were titrating a concentrated solution of sulphuric acid with barium hydrate, one could add the alkali without much reduction in the conductivity until a dilution to less than 15 per cent. had been reached; as each successive ion of SO_4 was combined with the barium and passed out of solution, another molecule of the acid would be at once dissociated, so that the active concentration would diminish but very slowly for a long time. And in an analogous manner we must consider that if a ferment were acting upon a concentrated solution of starch, as each active molecule became hydrolyzed its place would be taken by another molecule from the inactive excess, and thus the active mass would remain constant and the amount of inversion in the unit of time would remain constant. But in dilute solutions, as has been uniformly noted, the transformation is proportional to the mass—simply because under these conditions all the substrate is active in the reaction sense. Philoche[2] has recently reported upon a study of the acceleration of the saccharification of soluble starch by vegetable amylase, derived from malt and from the taka yeast. Employing solutions of 2 per cent. substrate, she found that for a time the constants rapidly descended, then became uniform and remained so during the experiment. It is possible that had the initial concentration of the substrate been lower, the constants

would have been uniform during the entire experiment, though the author states that the initial drop was observed in many series of digestions.

In a calculation of the relation of the mass to the reaction velocity in any reacting system, the total mass must be known to be identical with the active mass. This cannot be demonstrated for solutions of substances like starch and protein. For these stable or pasedo-colloids, we may in all probability assume that one of two conditions of state exist: (*a*) The substance is capable of a slight degree of true solution (just as a fat is); the larger part of the substance, however, is present suspended as a colloid. Under this interpretation, we should assume that the reaction is a function largely of the fraction in true solution, though the velocity would not depend upon it alone since the reaction occurs in the film of contact of the two phases, the watery and the colloidal phase. (*b*) The substance is incapable of any appreciable true solution. Under this interpretation there would be a reaction of equilibrium and partition between the colloid, the water and the catalysor. The present evidence tends to indicate that the laws of mass action, equilibrium, and partition tend to hold for the stable colloids, like starch and protein, but do not tend to hold for the typical unstable colloids, like the metallic hydrosols. Under either point of view, variations in the substrate concentrations would be expected to lead to variations in the intensity of the reaction; in the case of a suspended colloid because it would produce an alteration in the relation of equilibrium; in the case of a true solution because it would bring about an alteration in the active mass. That reactions may display great variations as the result of variations in concentrations, even in systems of simple inorganic reaction, is well illustrated in the recent observation of Sheppard. When hydroxylamine and silver oxide react in a system at moderate concentration, the reaction follows the equation $-2\ NH_2OH + Ag_2O = 2\ Ag + N_2 + 3\ H_2O$; when the system is in high dilution, however, the reaction follows the equation $-2\ NH_2OH + 2\ Ag_2O = 4\ Ag + N_2O + 3\ H_2$.

Since the velocity of the reaction of the fermentation of starch follows the common law $C = \frac{1}{t}\log\frac{A}{A-x}$ it is apparent that viewed from the point of view of the theoretical equation of

Henri to be described under invertase, the coefficients m and n must be equal, and thus the progress of the reaction is not deviated from the logarythmal curve.

Digestion experiments with amylase exhibit well the so-called false equilibrium of fermentations. After the transformation has come to a standstill, the reaction may be reinaugurated by the addition of more of the substrate or by the removal of the products (Morris & Glendenning). On account of the resistance of amylase to hydrolysis, these experiments can usually be repeated a number of times in one system. That the products depress the reaction through the relations of the mass law rather than by any chemical action upon the ferment may be shown by the following experiment. Two systems are prepared, one in a simple container, the other in a dialyser. The reaction in the dialyser will be more rapid and more fully completed than in the simple experiment. If now after the completion of the reactions in both, the sugar be removed from the simple test by dialysis, and both residues then tested for strength of ferment, it will be found that both have the same ferment content.

Relation of ferment concentration on acceleration of reaction. This has usually been stated by Pawlow to be proportional to the square root of the ferment, the results having been obtained with the Mett method. Henri[3] has found the transformation by vegetable amylase to be proportional to the ferment concentration, and I have obtained the same result with salivary amylase. Thus:

Substrate concentration one-quarter per cent. *a* has 1/10 vol. saliva; *b* has 1/5 vol. saliva. The times are the periods necessary to complete the given fractions of the total conversion.

	¼	½	¾
a (*min.*)	32	75	120
b	18	40	70

Brown and Glendenning,[2] however, found in their experiments with salivary amylase that the acceleration was proportional to the square root of the ferment concentration.

Influence of temperature. This is strikingly different for the processes of liquefaction and saccharification. The optimum temperature for the process of liquefaction lies high, between 60–70°,

for some preparations over 75°. At these temperatures, however, the ferment is rapidly destroyed, so that the time gained in liquefaction is more than lost in saccharification, and in practice, when such a high temperature is employed for liquefaction, a new quantum of ferment is added for the hydrolysis. The velocity for the hydrolytic acceleration rises in general conformity to the law for the increase of reaction velocity with temperature until 40°, from which point until 60° the reaction is very rapid, only to fall rapidly beyond 60°. Different preparations of amylase, however, vary widely in these regards. For the animal amylases the optimum temperature is some 15° to 20° lower for the reaction of hydrolysis, while, for salivary diastase at least, there is no marked difference in the optimum temperatures for the two processes. Solutions of amylase bear heating badly above 70°; dessicated preparations may be heated to over 100° for a long time without injury, though here again different preparations vary.

Reversion of reaction. The hydrolytic cleavage of starch, whether accelerated by acids or ferments, or whether accomplished by heat alone, is practically never completed. Starch does not remain in the system, but a certain proportion of dextrine remains. The amount of dextrine remaining depends to some extent on the concentration of the substrate; at high concentrations much more remains than at high dilutions. In a one-quarter per cent. solution not over 1 to 5 per cent. remains; at high concentrations as much as one-fifth may remain. Complete reactions have been reported as accomplished by the addition of fresh portions of ferment, but I have personally never been able to recover the full quota of maltose. The matter cannot be decided on the basis of a direct calculation of the amount of maltose to be derived from a known quantity of starch, since it is not known exactly how much maltose a unit of starch will yield, though the ratio is about 10 : 9. The demonstration of the incompleteness of the reaction is to be made by the demonstration of an increase in the maltose after the larger mass of the product has been removed from the system by dyalysis; if the ferment be not inactivated, this experiment succeeds. Since it is an obvious advantage in the mathematical interpretation of the results

to have the reaction as nearly complete as possible (*i.e.*, to have the station of equilibrium in starch = maltose as far to the right as possible, and the disturbing influence of the reversed reaction as small as possible), we have an additional reason for the employment of high dilutions of the substrate.

A clear reversion of the saccharification or of the liquefaction of starch has not been described. There exist, however, some studies by Wolff, Fernbach, and Maquenne that point strongly in this direction. They have constated after the action of diastase the reformation of coagulable starch and of a body giving the iodine stain. The authors themselves seem to incline to the view that the reversion may be the work of a totally different ferment. The matter is still under investigation, and should it transpire that true reversion has occurred, we may confidently believe the reversion to have been caused by the same ferment rather than by a different ferment. The formation of glycogen by reversion of ferment activity has been described by Cremer. He allowed a yeast amylase, free of glycogen, to act upon a concentrated solution of glucose. A polysaccharide was formed that gave the qualitative reactions of glycogen. It may not have been identical with glycogen, but it was a starch-like body, and with that the synthesis of a polysaccharide by acceleration of reversion through ferment action was accomplished. We may in all probability regard the formation of glycogen from sugar as an instance of the formation of a poly-saccharide from a monosaccharide by enzymic acceleration of the reversed reaction. That acid reversion of the hydrolysis exists has been made very probable by Wohl, who has further shown that under conditions of high concentration of the substrate the acid hydrolysis is always a limited reaction.

Conditions of activity. Amylases of vegetable origin seem to operate more rapidly at a slightly acid reaction, though fermentation occurs actively at a neutral reaction. The animal amylases, on the contrary, operate in a faintly alkaline or neutral reaction better than in an acid reaction. According to Foa, the alkalinity of the saliva is about N/80000. For both classes a slight excess of the alkali or acid is harmful, and the optimum concentration seems to be rather narrow. The influence of the

reaction, however, varies notably with the temperature, as well as with the concentrations of the substrate and the ferment. Of quantitative data there are none of value. In all the published work on the degree of acidity and alkalinity employed in the experiments with amylase, that degree of reaction has been determined either by adding a measured quantity or by an end titration. The procedures of course do not give information upon the point desired, the concentration of hydrogen or hydroxylion ions. The action of acids and alkalies in the concentrations employed is in the rôle of a zymoexciter, not that of a second catalysor whose acceleration is added to that of the ferment. Under the circumstances we do not know whether it may not be in part the undissociated molecule that acts as the zymoexcitor; just as many other undissociated substances may thus act. The entire situation is unclear; but it is certain that none of the experiments ever determined exactly what active acidity or alkalinity was being used. This accelerating action of acid for vegetable diastase seems to be confined to the saccharification, and does not affect the process of liquefaction.

Many other substances depress the activity: salts of lead, mercury, barium, arsenic, iron, alcohols, phenols, formaldehyde. Chloroform has little influence; toluol has none. Stimulating, on the other hand, are the phosphates of calcium and ammonium, the common alumns, aluminum acetate, and asparagin. With the exception of the asparagin, these substances act largely upon the process of liquefaction. Amido acids as a group stimulate the action of amylase, while acid amides depress effront. There are well defined optimum concentrations for these substances. For both the stimulating and the depressing substances the action is in a general way inversely to the mass of ferment present. There is an enormous literature upon the subjects of the action of various substances on amylase. Though different experimenters have carried out elaborate series of experiments, their results have been often very contradictory, both in the quantitative and in qualitative details. The reason for this lies in the fact that the reactions of a ferment to different substances and the reactions of various substances upon a ferment are to a marked degree different with different preparations of the ferment, just as

are its resistances to heat, to hydrolysis, etc., relations that are well known to hold for all colloids, for the metallic colloids as well as for ferments. Under these circumstances, investigations on the actions of different chemical substances on amylase possess an approximate value only.

Amylase is more rapidly destroyed by being heated in simple solution than when heated in the presence of starch. Under favorable conditions of temperature, concentration, and reaction, amylase is destroyed to but a very slight extent during the course of a fermentation; it is so resistant to hydrolysis that it may be said to display for practical purposes the integrity characteristic of a true catalysor, that it shall not be altered by the reaction it accelerates. In pure solutions the ferment is destroyed with moderate rapidity; when dessicated it may be kept indefinitely, and is then resistant to high temperatures. The best method of preserving amylase is to keep it mixed with starch at a low temperature; under such conditions one can prepare enough for a series of experiments, and very little change will occur.

Chemical properties. The chemical attributes of amylase are not well known. There has been apparently only one preparation of a reasonably pure amylase of vegetable origin, by Wroblowsky, and none of animal amylase. The substance isolated by Wroblowsky was colorless, very soluble in water, of neutral reaction, not coagulable by heat, was precipitated by a 50% ammonium sulphate and by the salts of lead and mercury, responded to the biuret, Millon and Xanthoproteic reactions, and was precipitated by tannic acid and alcohol without denaturation. It is not crystalline, very slightly diffusible if at all in the practical sense, but may be filtered (though with loss) through infusorial filters. It clings to colloidal structures, and cannot be filtered through paper without some loss. It contains some 16.5 per cent. of nitrogen, and is digestible by pepsin, less readily by trypsin. The products of the digestion are amido acids, leucine, tyrosin and arginine having been isolated. In general terms it may be named an albumose.

INVERTASE AND LACTASE.

The inversion ferment for cane sugar was discovered in beer yeast by Doebereiner and Mitscherlich; it was first isolated by Berthelot. It is now known to occur almost universally. In the higher animals it is present in the saliva, in the secretions of the small intestines, and in the liver. It seemingly displays a less wide distribution in the animal kingdom than amylase, probably because it has been less often sought for. Cohnheim found it present in all echinoderms examined. It has been found in the eggs of crustaceans. In the vegetable world it is probably the most widely distributed of ferments. Unlike the amylase, it is found to a less extent in the seeds and to a greater extent in the growing parts, buds, and leaves. Kastle and Clark in a recent study examined nineteen species belonging to fourteen families and found invertase in all. It is often found in tissues that possess no sacchrose. It is found frequently in bacteria, not only in the yeasts but also in the bacilli and cocci. The bacteria that have the function of lactic acid fermentation seem to be able to accelerate also the inversion of sacchrose. Among the disease-producing micro-organisms the following possess in a prominent manner the faculty of inverting cane sugar and then fermenting the hexose: Pneumo-bacillus of Friedlaender, Bac. diphtherie, Bac. malignant oedema, the colon bacillus, and the proteus vulgaris. Doubtless there are many others. The named micro-organisms are also able to invert milk sugar, but have no power to accelerate the inversion of maltose.

Whether these various invertases are identical is not known. Of all ferments, invertases from different sources display the most notable variations in physical and chemical qualities. Different invertases display variations of as much as 40° in their optimum temperatures; some are very sensitive to acids and heat, others quite resistant; some are dextro-rotary, others do not polarize light; some solutions are very colloidal, others not at all so; some preparations are diffusible, others scarcely filterable. Very interesting are the differences in the optimum temperatures of the two common yeasts of beer; the invertase of surface-fermentation yeast has an optimum temperature of some 20–30°

higher than that of sediment-fermentation yeast; the one is derived from a yeast with strong predeliction for oxygen, the other from a yeast with a slight predeliction for oxygen, and nevertheless these yeasts represent cultural deviations from one parent stock. Invertase from one source also will display marked variations in properties, depending upon differences in the history of the culture and the methods of preparation. These facts indicate the futility of present speculations on the unity or multiplicity of invertases. It is known that invertase is able to accelerate the inversion of sacchrose alone, not of lactose or maltose. Maltase is apparently of less wise distribution, though it is encountered largely in grains, yeasts, and in the growing parts of plants. It was discovered by Gusenier, though the cleavage of maltose in urine had been described by Bèchamp. In mammalians it has been shown to exist in the saliva, pancreatic juice, succus entericus, liver, blood, and urine. The current statements that maltase is able to act upon starch and glycogen is surely an error based upon an admixture of amylase. Lactase, the ferment of milk sugar, was first described by Beyerinck, who found it in the kephyr yeast. It is now known to be present in many yeasts. It has not yet been directly shown to be present in animals apart from the intestinal secretion, but the mere fact that lactose is inverted in the body indicates its presence there. Lactase has been found in the foetal intestinal tract. (Brochin.)

Maltase, the third of the prominent inversion ferments, is very widely distributed in nature, being an almost invariable accompaniment of amylase in plants, yeasts, and bacteria, and existing also in some lower forms of vegetable life that possess no amylase. Maltose is present in the salivary secretion, the pancreatic juice, the succus entericus, liver tissue, the blood, and thus in all organs of the mammalian body. That a particular yeast almost never contains both lactase and maltase, as pointed out by Fischer, is a good illustration of adaptation to the external medium.

The reaction of inversion. The hydrolysis of the disaccharides is a direct addition of water and cleavage into the two components.

$C_{12}H_{22}O_{11}$	+	H_2O	=	$C_6H_{12}O_6$	+	$C_6H_{12}O_6$
Sacchrose	+	water	=	d-glucose	+	d-laevulose.
Maltose	+	water	=	d-glucose	+	d-glucose.
Lactose	+	water	=	d-glucose	+	d-galactose.

For cane sugar the reaction would run:

$$O\begin{cases}CH.CHOH.CHOH.CH.CHOH.CH_2OH \\ CH_2.CH.CHOH.CHOH.COH.CH_2OH\end{cases} + H_2O = \begin{cases}CHO(CHOH)_4CH_2OH \\ CH_2OH(CHOH)_3CO.CH_2OH\end{cases}$$

So far as we know there are no sub-stages in the reaction. The acid hydrolysis yields the identical products, and there are no known qualitative differences in the reactions. The hydrolysis occurs in pure water, though very slowly, rapidly in steam. This auto-hydrolysis has been studied recently by Lindet. Solutions of pure cane sugar, and of the two products, conduct a current somewhat better than the same water in which they were dissolved, and from this Lindet infers a slightly acid character in sugars, or in betters terms, a slight dissociation. Experiments in glass he found negative on account of the alkali from the glass; the different metals affected the hydrolysis in different degrees, some depressing, some accelerating, while some were of no effect. The auto-hydrolysis was accelerated by the addition of the two hexoses that represent the products. The actual agent in this auto-hydrolysis, of which every inversion must be looked upon as the acceleration, is to be sought in the hydrogen ion of the dissociated water. According to the conception of catalytic reactions that Euler has endeavored to introduce into the world of organic chemistry, the sugar must also be looked upon as subject to a slight degree of electrolytic dissociation. This dissociation may be conceivably either into a dextrose-ion and a laevulose-ion, or into a cation $O_{12}H_{21}O_{10}^{+}$ and an anion OH^{-}. According to such a conception, the conditions in a solution of sugar in water correspond to a system of four active ions in very high dilution. Euler is inclined to consider that the more probable of the two possible dissociations is the latter, and that the hydrolysis is determined by the concentration of the saccharose cation.

It must be clearly understood that the hypothesis of the ionic dissociation of cane sugar is practically devoid of experimental basis, and represents little else than the expansion of the idea that all reactions are ion reactions to the domain of organic chemistry. For the practical study of the complicated problem of the catalytic acceleration of the hydrolysis of a sugar, the hypothesis presents no obvious advantage. It has up to the present been impossible to demonstrate ionic dissociation in the vast number of organic substances in the sense in which the law of Arrhenius may be shown to hold for inorganic substances. That all reactions, in whatever medium they occur, are ion reactions, cannot be maintained today. The experimental problem in the fermentations is already difficult enough, and it is neither simplified nor elucidated by the introduction of the hypothesis of Euler.

The members of the platinum group all act as accelerators to the acid inversion of sugar. They accelerate the auto-hydrolysis as well as the acid hydrolysis, acting for the latter especially as zymoexciters rather than as additional catalysors. Plzak and Husek have shown that for the members of the whole platinum group zinc and manganese act as negative catalysors. I have determined that platinum is not a zymoexciter for invertase, and if it act at all as an accelerator under such circumstances that action is so weak as not to appear in the final results.

In addition to its faculty of acceleration of the hydrolysis of cane sugar, invertase possesses the faculty of accelerating the hydrolysis of certain complex sugars. Gentianose, a complex carbohydrate existing in Gentiana lutea, consists of two molecules of d-glucose and one molecule of d-laevulose. Invertase has the faculty of splitting off one molecule, that of d-laevulose, leaving a disaccharide remaining, gentiobiose, composed of two molecules of d-glucose. This gentiobiose, though composed of two molecules of d-glucose, is not identical with maltose in its chemical and physical properties, and is not fermentable by maltase, but is fermentable by emulsin. In a similar manner invertase is able to accelerate the partial hydrolysis of another complex sugar, melitriose, a carbohydrate composed of a molecule each of d-glucose, d-galactose, and d-laevulose. Invertase will ferment melitriose in the sense that it will split off the molecule

of d-laevulose, leaving a disaccharide, melibiose. This melibiose is composed of one molecule of d-galactose and one of d-glucose, but it is not identical with milk sugar; it has different physical and chemical properties; it is not fermentable by lactase, but is fermentable by emulsin and by a ferment present in certain yeasts, that again will not ferment milk sugar. (Bourquelot, Fischer.) Invertase is able, furthermore, to accelerate the hydrolysis of the synthetic *a*-methyl-fructosides of Fischer (not the *b*-fructosides), though unable to influence the *a*-methyl glucosides of d-glucose or d-galactose. These several facts seem to ally the acceleration-faculty of invertase to the presence of d-laevulose in the disaccharide. This is of especial interest when taken in connection with the fact that of the products of the reaction of inversion it is apparently the d-laevulose alone that exerts the function of the law of mass action on the progress of the reaction of hydrolysis; if the d-laevulose be removed from the system, the d-glucose does not depress the progression, while, on the other hand, the removal of the d-glucose alone is entirely without effect. This suggests apparently a fundamental distinction between the accelerations of acid and invertase on the hydrolysis of sugar. The distinction may, however, be only quantitative, and not qualitative; exact investigations are needed here. Weak acid is able to effect only the first stage of the hydrolysis of gentianose and melitriose, the splitting off of the d-laevulose, analogous to the action of the invertase; strong concentrations of acid will, however, effect a complete cleavage.

In an analogous manner maltase seems to act up the *a* series of d-glucose, while lactase acts upon the *b* series of d-glucose and d-galactose.

Kinetics of the reaction. A peculiar interest attaches to the study of the kinetics of the fermentation of cane sugar, since it was with this body that Wilhelmy first established the law of reaction bearing his name. It has naturally attracted much attention, since it is one of the most feasible of fermentations with which to work; the substrate may be procured in a state of high purity, the ferment is easily obtained, and the measurement of the reaction with the polarimeter is simple and accurate.

O'Sullivan and Thompson first studied the question in a

quantitative way. They believed that their results could be represented by a logarythmal curve, but a closer inspection of their figures shows that there is a gradual increase in the figures for the constants as the reaction progresses. Tammann about the same time showed that the reaction with invertase is not a complete reaction as in the case of an ordinary acid inversion. While his figures indicate in general a rough conformation to the law of acid inversion, there were many deviations. These Tammann sought to attribute in part to the differences in the relations with varying amounts of ferments; with high concentrations of ferment the reaction seemed to lag behind; with lower concentrations it seemed to rush ahead.

Duclaux next studied fermentative inversion. He began the study with several preconceptions that seriously influenced his work, but in other ways his methods were so superior to those of the contemporaneous biological world that he is to be regarded as the father of accurate biological study of fermentation. He employed in large part high concentrations, and although in his tests with low concentrations the velocity was proportional to the mass of the substrate, the fact that at high concentrations the reaction seemed independent of the mass of substrate led him to reject the doctrine of mass action. He held that in the beginning the reaction exhibited a regular progression, that the quantity of substance transformed was proportional to the time, $\frac{dx}{dt} = C$ (thus corresponding to a straight line), and that this condition persisted until about one-fifth of the sugar was inverted, when the reaction became lessened owing to the influence of the products of reaction. From this point on he regarded the reaction as being represented by the equation $C = \frac{1}{t} \log \frac{A}{A-x}$. He studied in detail the retardation of the products upon the progress of the reaction. This retardation he conceived as depending not upon the mass of the products themselves, but in their relation to the initial concentration of the substrate, and this relation he defined for each moment in the reaction to be $\frac{x}{a}$. Obviously this manner of conception is entirely out of harmony with the law of mass action, as well as with the kinetic considerations concerned in a reaction of reversibility, and indeed Duclaux specifi-

cally rejected the possibility of a reversion of ferment action upon our present basis of thermodynamics.

A. N. Brown confirmed the previous statements that in high concentrations a constant unit of transformation occurs in a unit of time, and that later the results proceed with greater rapidity than could be accounted for in a simple logarythmal curve. He also confirmed the observations of Duclaux upon the retardative action of the products of fermentation.

Within recent years the kinetics of the reaction of inversion of cane sugar have been thoroughly worked through by Victor Henri.[4] He employed pure substances and worked under carefully controlled conditions of concentration, temperature, etc.; and most of all, he proceeded upon an entirely clear definition of the actual problem. The figures he obtained for the constants according to the equation $C = \frac{1}{t} \log \frac{A}{A-x}$ exhibited a regular and progressive numerical increase. Thus in four different experiments the figures for the constants rose from 25 to 33, 57 to 88, 58 to 96, and from 105 to 185, increases more regular and pronounced than had been previously observed. Employing the Ostwald method for the calculation of a catalytic auto-catalysis, Henri obtained an empiric equation, which when integrated reads: $2\, k_1 = \frac{1}{t}\, l\, \frac{A+x}{A-x}$. When calculated with the aid of this equation, the new constants exhibited a satisfactory uniformity. Henri further confirmed the observations that invertase is not appreciably inactivated during the course of a fermentation of moderate length. This he accomplished by adding new sugar to the system, and observing that on the restoration of the original concentration the original velocity of the reaction was restored. He found that with dilute solutions the reaction was in general terms proportional to the mass of substrate, though this held but to quarter normal solutions. Nevertheless the velocity of the reaction in the favorably dilute solutions did not hold pace exactly with the substrate concentration, but depended in part upon the concentration of the products. As a rule he found that the lower the concentration the higher the constant. The acceleration of the reaction he found quite closely proportional to the quantity of ferment.

This empiric equation of Henri $-2K = \frac{1}{t} l \frac{A+x}{A-x}$ was of very doubtful value. In the first place, while the constants obtained through its use exhibited fairly uniform concordance in a particular experiment, they were not at all concordant in different tests with different substrate concentrations. They exhibited in short the same relations that were described for the constants of the amylase reactions. Secondly, it is certain that there is not an auto-catalysis in the inversion experiment; the products depress, they do not stimulate the reaction of the fermentative acceleration. That the constants increase as the concentration of the products increase is true, but this is not to be interpreted to indicate an auto-catalysis, contradictory as this statement may seem to be, because the direct experiment shows the products to be negative. The facts suggested that the increase in the constants was associated with some alteration in the intensity of the enzymic action; the enzymic acceleration was interpreted to depend not strictly on the ferment concentration, but also on the substrate and product concentrations. Henri next attempted to define these relations, and his studies on this point constitute really the first solid beginnings of a theory of the dynamics of fermentation.

Henri based his theories on the assumption that the catalytic and fermentative accelerations rest chemically on intermediary reactions. The ferment may be obviously in combination with the substrate, with the products or free. It is assumed by Henri that all three states exist; that the combinations with the substrate and products are not complete and are subject to the law of mass action. Obviously therefore, as the fermentation proceeds the relation of the combinations with the substrate and the products respectively is being constantly shifted. Since these considerations promise to be of much importance to the future developments of fermentations, we shall consider them in detail, the more as the brochure of Henri[5] is difficult of access.

In the chemical system under consideration, A is the substrate, the saccharose; F is the ferment, x the sugar inverted in the time t, and A-x the amount of substrate in the system at the end of the time t. Under the assumption that the ferment F is divided into three parts, that combined with the substrate we will

term Fs, that with the products we will term Fp, and the free ferment we will term Ff. These combinations are to be considered in equilibrium, in accordance with the law of mass action. The total ferment mass is then $F = Fs + Fp + Ff$. The equilibrium between the ferment and the substrate is expressed in the equation: $Ff\ (A - x) = \frac{1}{m}\ Fs$. The equilibrium between the ferment and the products is expressed in the equation: $Ff\ x = \frac{1}{n}\ Fp$. m and n are the constants of equilibrium. The amount of ferment thus free and combined may be then calculated. Thus $Ff = \frac{F}{1 + m(A-x) + nx}$ and $Fs = \frac{m.\ F.\ (A-x)}{1 + m\ (A-x) + nx}$. The acceleration may be related either to the ferment combined with the substrate (Fs) or to the ferment free in the system (Ff). If the acceleration be related to the ferment in combination with the substrate (Fs), the differential equation representing the reaction would be $-\frac{dx}{dt} = C.\ Fs$; replaeing now the Fs with the equation for it as given above, we have: $-\frac{dx}{dt} = \frac{C.\ m.\ F.\ (A-x)}{1 + m\ (A-x) + nx}$. If, on the other hand, the acceleration be related to the ferment free in the system, it would be proportional to the free ferment and to the substrate, and the differential equation to express this relation would be $\frac{dx}{dt} = C.\ Ff.\ (A - x)$; when the value of Ff as given above is inserted, we have: $-\frac{dx}{dt} = \frac{C.\ F.\ (A-x)}{1 + m(A-x) + nx}$. These two equations are identical; it is therefore immaterial to the mathematical development of the situation whether we assume the active mass of the ferment to be that related to the substrate or free in the system. The considerations based on the equilibria between these several relations leads us then to substitute for the simple equation $\frac{dx}{dt} = C\frac{A}{A-x}$ the equation $-\frac{dx}{dt} = \frac{C_3\ (A-x)}{1+m(A-x)+nx}$, in which C_3 is a constant proportional to the quantity of ferment, m and n constants that characterize the relations of the ferment to the substrate and products respectively (they will vary with alterations in the medium and with temperature), A is the initial concentration of substrate expressed in normal terms, x is the substrate inverted in the time t. For 25°, with solution of not over half normal, Henri found m and n to be respectively 30 and 10. When the above equation is integrated under the

assumption that when $t = 0$, $x = 0$, we have: $C_3 = \frac{A}{t}\left[(m-n)\frac{x}{A} + n.\,1\frac{A}{A-x}\right] + \frac{1}{t}1\frac{A}{A-x}$. When the values for m (30) and n (10) are inserted into the equation, we have: $C_3 = \frac{10.A}{t}\left[2\frac{x}{A} + 1\frac{A}{A-x}\right] + \frac{1}{t}1\frac{A}{A-x}$. This is the equation employed in the calculations. The equation assumes, apart from strictly controlled conditions of experimentation, the non-inactivation of the ferment, the non-reversion of the reaction, and the absence of auto-catalysors. The drawback of the equation lies in the presence of three constants; equations with three constants approach closely interpolation equations; the m and n ought to be determinable experimentally, or at least controllable. The values given for m and n are not accurate, only approximate. One can see from the figures of Henri that the agreement in the constants would be closer were m relatively somewhat greater. With this equation Henri has calculated the results of a large number of experimental findings. The C_3 is fairly concordant in the same series, and also, what is of more importance, closely concordant in different series with varying substrate concentrations. The equation therefore appears to so closely express the intensity of ferment activity that taken into connection with the relations of concentration in the system, results are obtained that are in close accord with the law of mass action. The equation of Henri is in reality nothing else than his equation for the intensity of ferment action $I = \frac{C}{1 + m(A-x) + nx}$ inserted into the equation for a monomolecular reaction. Henri studied maltase, and found the constants to exhibit a curve; first they rose and later fell. There was, however, no regularity in the curve.

Barendrecht in his recent paper attempting to explain the nature of fermentation as the result of emanations, reported upon experiments with invertase. His figures quite closely resemble those of Henri.

Visser has recently published a study of the reaction of fermentation based on the hypothesis that the deviation in the results from the common law is due to variations in the intensity of the ferment action, and this he tries to relate to the concen-

trations in the system in accordance with the law of mass action. He first attempted to include in his considerations the fact that the reactions of fermentation are reversible, and grounded an equation on the relations of the constants of equilibrium. The reaction he represented in the equation $-\frac{dx}{dt} = C_1 (A - x) C_2 x^2$. When this is integrated under the assumption that when $t = O$, $x = O$, we have: Const. $= \frac{1}{t} l \frac{(A-b) \quad ((A-x)-a)}{(A-a) \quad ((A-x)-b)}$. b is the concentration of the substrate in the stage of equilibrium, and a may be calculated when b is known by means of $ab = A^2$. These constants did not give good agreement for the experimental results. A consideration of the facts will indicate that this could not have been expected. While it is true in the theoretical sense that the reaction of inversion is reversible, in practice it is not so because the station of equilibrium is so near the point of a complete reaction. When a solution such as is used in inversion experiments is in equilibrium, we have sacchrose 1 : products 99. Obviously therefore the constant for the reaction in the direction of the left, the $<-C$ in the equation, must be very small in comparison to the constant for the reaction in the direction of the right, the $C->$ of the equation. Under these circumstances, the equation as a matter of fact is almost identical in its mathematical valuation to the common equation for a monomolecular reaction. This last equation, however, it will be recalled, does not give concordant constants; the figures rise progressively. So do they rise progressively if the equation of Visser is used. Furthermore, the apparent station of equilibrium cannot in our present state of knowledge be entirely identified with the station of equilibrium in a reaction in a simple system like ester + water = alcohol + fatty acid. Knoblauch has shown that the constant of equilibrium $= \frac{C->}{<-C}$, and when the system is in a state of equilibrium the transformation in the one direction exactly equals that in the other. This is in direct accord with the theory. Now when in an inversion experiment the reaction ceases in the situation sugar 1 $<=>$ products 99, it is to be assumed in accordance with the theory that in each moment as much sugar is being reformed as there is sugar being hydrolyzed. But it is possible to show experimentally that this is not the case. Experiments in the

reversion of ferment reactions indicate positively that it will require months to reform the amount of sugar that may under the same conditions be hydrolyzed within a few hours. While the theoretical equilibrium is without doubt contained in the apparent equilibrium observed, some other factor or factors are also contained therein, and one of these factors is unquestionably related to the ferment itself. These considerations indicate that the use of the theory of the reversibility of the reaction of hydrolysis in the equation for the reaction velocity as proposed by Visser could not have been expected to lead to concordant results; and the practical findings are in full accord herewith.

Visser next attempted to define the intensity of ferment action. This he did in a purely empirical manner. Upon the assumption that the intensity of ferment action is related to the concentration of the substrate and of the products, he found: Ferment intensity (I) $= \frac{Const.}{4A^2+2A(A-x)+(A-x)^2}$. This he inserted into the previous equation $-\frac{dx}{dt} = C_1 (A-x) C_2 . x^2$. Since the value of this equation is so nearly that of the old equation, the latter, being much simpler, may be employed. This will yield $-\frac{dx}{dt} = C (A-x) I. = \frac{dx}{dt} C_1(A-x) . \frac{C_2}{4A^2+2A(A-x)+(A-x)^2}$. When integrated on the assumption that $x = 0$ when $t = 0$, we have: $2 C_1 C_2 . t = \Big(5A+(A-x)\Big)\Big(A-(A-x)\Big) + 8A^2 \, l \frac{A}{A-x}$. The results are fairly concordant in the different measurements of the same series, but not at all concordant in the different measurement of series at varying concentrations. In this respect they are without the validity that is attached to the constants when calculated according to the equation of Henri.

Herzog has applied to inversion the Nernst theory of reaction velocity in a heterogeneous system. It will be recalled that in the Nernst presentation stress is laid upon the fact that such stirring must be provided that the relations of concentration in the film of boundary contact of the two phases provide for a very thin layer in which the velocity of diffusion then becomes the determining variable, the velocity of chemical reaction being assumed to be of practically infinitesimal velocity. This stirring must be such as to obliterate the effects of the convection streams;

in a word, the concentration of the solution in the system must be everywhere the same, and under these circumstances the only diffusion velocity variable, and therefore measurable, will be located at the boundary of contact of the two phases. In the application of this proposition to a system in a state of fermentation, Herzog regards the heterogeneous system as comparable to a capillary system, in which the capillary walls are formed by the surfaces of the colloidal particles of the ferment in the case of the inversion of sugar, of both the ferment and the substrate, however, in the case of the fermentation of protein. Variations in density will result in the setting up of circulating currents through the capillary spaces. The velocity of this circulating stream, however, will depend on the viscosity, the internal friction, of the system. The higher the viscosity, the slower the currents; and the slower the currents, the less the unit of substrate that will be carried to the surface of the ferment in the unit of time. In this proposition are two assumptions. It is assumed firstly that the surface dimensions of the colloid, the ferment, are constant; that the dimensions of the contact of the two phases are constant; variations in this are recognized by Herzog as leading necessarily to an auto-catalysis. It is assumed secondly that the thickness of the boundary film is maintained constant, that the capillary currents fully realize the conditions of the Nernst hypothesis. These being granted, the reaction velocity (*i.e.*, the diffusion velocity) may be taken to be an exponential function of the viscosity, and an equation is feasible. This reads: $C = \left(\frac{1}{n}\right)^m$, in which n in the function of the viscosity and m a constant that may be determined by the equation of Rudorf.

The viscosity of certain solutions may, according to Rudorf, be determined from the concentrations according to the equation $n = R + Aa + Ba^2 + Ca^3$, or better under the proposition that the viscosity of water = 1: $n = A + Ba + Ca^2 + Da^3$. n is the internal friction, A the viscosity of water (1), a the concentration, and A, B, C, and D constants. On the assumption that $R = O$, we then obtain $C = \left(\frac{1}{(Aa + Ba^2 + Ca^3)}\right)^m$. m is determined by calculation to be one-half. A, B, and C must be determined for each different ferment. The results of the cal-

culation of the experimental findings are fairly satisfactory, the constants are quite concordant.

Henri[5] has pointed out that the equation of Herzog is in contradiction to the diffusion law of Fick. Herzog assumes $R = O$, which would need to be demonstrated. Henri has also pointed out that the actual viscosities of the sugar solutions are not at all identical with the postulated viscosities as calculated from the constants *A*, *B*, and *C*. To my mind, the most direct reflection on the equation of Herzog is contained in the fact that the addition of such amounts of d-glucose and d-laevulose as possess the same internal friction should, according to the hypothesis, exert the same influence upon the reaction velocity, whereas as a matter of fact d-laevulose is potent, while d-glucose is practically without influence. The velocities of different fermentations vary enormously under conditions in which such variations in diffusion cannot be assumed to occur. Negative to the idea is also the fact that within certain limits the larger the proportion of substrate to ferment the closer the theory is observed. That trypsin displays the same behavior when digesting protamine, scarcely colloidal, as when digesting the coarse suspensions of coagulated pulverized egg albumin, is also not to the favor of the Herzog hypothesis. Herzog has himself pointed out that an auto-catalysis is almost inexplicable under this theory. The acceleration of velocity by increase in temperature is also out of harmony with the diffusion hypothesis. Herzog does not himself regard his equation as more than an orientation interpolation equation, and while the insufficiency of the equation as representing the progression of a fermentation is to be admitted, the fact remains that it deals with an aspect of the problem, the condition of the heterogeneity in the reacting system, that had been hitherto overlooked. And while it may be admitted that the dominant variable may not be located in the relations of this heterogeneity, an influential variable is certainly present there. This statement is illustrated by known facts in the domain of colloidal metals. The catalytic action of colloidal platinum, for example, is not a function of the mass of platinum, but rather of the fineness of its subdivision into colloidal particles. Different preparations of colloidal platinum of the same mass percentage vary

widely in the colloidality of the suspension; some are almost amorphous, others are typically colloidal. Now the catalytic activity of such suspensions is in general terms proportional to the colloidality of the preparation. The actual acceleration may be confidently assumed to lie in intermediary reactions in which the metal acts in chemical combination. But the effectiveness of these intermediary reactions is closely allied to the colloidality of the suspension of platinum.

As a matter of fact, the theory of Nernst ought not to be judged by this application, the provisional nature of which has been emphasized by Herzog himself. The equation of Rudorf was not designed for the present type of calculation. Rudorf determined the relation between the concentration and viscosity of solutions of acetic acid, urea, sugar, tartaric acid, propyl alcohol, and acetone in water. He found that neither the older exponential equation of Arrhenius ($n = A^x$) nor the linear equation ($n = 1 + an$) corresponded to the actual relations. With the simple solutions employed by him he found that the molecular relations in the system were never constant; if the substance underwent polymerization, the deviations tended to be proportional to n; if combination occurred between solvent and solute, the deviations tended to be proportional to n^2 If an electrolyte were present in the system the relations would be very complex, and Rudorf studied no such system. If two substances were present, the relations would be much more complex, even were they both undissociated, and Rudorf studied no such system. He studied solutions of urea, sodium bromide, and hydrochinon in acetic acid, but the results were not interpretable under the equation. Now Herzog takes the equation that was erected to express the relations between the concentration of a pure organic substance in water and the viscosity of the solution, and applies it to a complex system, in the attempt to relate its internal friction to its concentration. The fermentation system contains several, indeed many substances, both organic, dissociated, and electrolytic. There is in the work of Rudorf no warrant for this use of the equation; it was not erected with such conditions in view and is not adapted to account for them. Rudorf gives as the causes for the deviations of the results from the linear equation

the following factors that operate in the viscosity of a solution. *a*. The electrical situation, whether dissociated or not. *b*. Loose combinations between solution and solute. *c*. Combinations between the molecules of the solute, polymerization. *d*. Electrostriction, resulting from the charging with ions. In the case of a fermentation system, we would have in addition the combinations between the different components in the system. It may be objected that apart from the substrate and the water, the other components in a fermentation system, ferment, salts, and other coincidental organic substances, are present in so small an amount as not to disturb seriously the relations of the solute and the solvent. To this it must be replied that only the experiment can answer to what extent deviations are produced. Such experiments were not carried out by Rudorf, such conditions were not contemplated by him, and the application of the equation to conditions for the interpretation of which it was not devised is an improper procedure. The work of Dunstan illustrates the anomalies in the viscosities of mixtures, and finally Fawsitt has recently shown that some of the viscosity measurements of Rudorf were inaccurate.

Armstrong[1] has very recently carried out accurate studies upon the fermentation of milk sugar, based upon the same proposition that during the course of a fermentation there are combinations between the ferment and the components of the reaction system. He too found that when the substrate was in high concentration the velocity was not proportional to the mass of substrate, though it could be made so by the employment of huge quantities of ferment. Diluted solutions, on the contrary, exhibited a velocity proportional to the mass of milk sugar. In solutions of high concentration the curve was for a short time linear, then became logarythmal, and towards the close again very irregular. Armstrong therefore divides the reaction into three stages: in the first the transformation is a function of time, in the second the velocity is a function of the concentration of the substrate, in the last the regular progression of the hydrolysis is disturbed by the action of the concentrated products. With lower initial concentrations only periods two and three are observed. The equation of Henri agreed very well with the results,

except for the last period. The products of the reaction were found to be actively depressant, and this retardation was caused solely by the d-galactose. This corresponds directly with the analogous findings of Henri that laevulose alone depresses invertase. This depression of the action of the lactase was not due to its destruction; on the contrary, the ferment seemed very resistant to hydrolysis.

Armstrong[2] also studied the acid hydrolysis of milk sugar in order to compare it with the fermentation. He found very analogous conditions. With a high concentration of the substrate and a low concentration of the acid the early reaction follows a linear course; later the logarythmal curve appears. At lower substrate concentrations and with a greater acid concentration the curve is logarythmal from the start, except that the progress is somewhat in advance of the theory. The velocity is very much slower than for the ferment, something like 1000×1. He considers that the two inversions are quite alike, and that the differences are due largely if not entirely to the greater affinity of the ferment for the sacchrose as contrasted with that of the acid, and to the colloidal nature of the ferment.

Armstrong[3] also studied the inversion of cane sugar, and reached results that were in general in harmony with those of Henri.

Relation of ferment mass to acceleration of reaction. In practically all the published studies in which this question has been investigated it has been found that the acceleration was proportional to the mass of ferment in the system. This is well illustrated in the figures of Henri and Visser. It holds true only for dilute solutions, the same concentrations of the substrate, in fact, that yield the best results in investigations to determine the relation of velocity of transformation to the mass of substrate. It is most interesting too to observe that it is only within this range of concentrations that the law for increase of reaction with increase of temperature holds good.

Reversion of reaction. The transformation of sacchrose into the hexoses is never completely accomplished by invertase. In weak solutions, such as are commonly employed in the investigations, the untransformed sugar does not amount to over 1 or 2

per cent. In more concentrated solutions the unconverted portion may be as high as 5 or more per cent. While therefore the actual amount left unconverted is small, it may be easily measured on account of the delicacy of the polariscopic method employed. It is possible therefore to show that the addition of further sugar after the reaction has passed into a state of equilibrium will result in the augmentation of the inversion until the state of equilibrium has been again established. If, on the other hand, the products be removed, the remaining substrate will be for measurable purposes completely inverted. That the active agent in the reversed reaction is the mass of d-laevulose, and not the mass of d-glucose, was first demonstrated by Henri, and it is easy of confirmation. This is clearly shown in the following experiment. Four systems are prepared. In *a* we have a standard solution of sugar and ferment; in *b* the same plus a unit of d-laevulose; in *c* the same plus a corresponding unit of d-glucose; in *d* the same standard solution plus an amount of invert sugar containing the d-laevulose of *b*. The sugars must be pure in the steriosomeric sense. The results of the experiment are that the velocities of *a* and *c* are alike, the velocities of *b* and *d* alike, and much lower than *a* and *c*. Henri has further determined that the inhibitory action of the d-laevulose is approximately proportional to its mass if the substrate concentration is constant; that with a fixed mass of invert sugar the depression is in general inversely proportional to the concentration of the substrate; and that when both are fixed with relation to each other the retardation is proportional to the dilution of the system. These facts are of especial interest. Not only does the actual hydrolysis of the cane sugar under the acceleration of the invertase vary with different substrate concentrations disproportionately to the law, but an adventitious variable varies in its disturbing action with changes in the concentrations.

Reversion of enzymic activity has been accomplished for the inversion of sugar; it was here in fact first accomplished. Wohl and Fischer first determined that when sulphuric acid is allowed to act upon concentrated solutions of glucose a disaccharide was formed which they were able to identify as isomaltose. This is obviously the converse of the common result that acid

will not entirely invert concentrated solutions of saccharose. Hill attempted the reversion by the use of a yeast that was incapable of fermenting glucose. After this yeast had been for some time in contact with a concentrated solution of glucose, the solution was found to have acquired an increase of optical rotation and to have suffered a loss in the power of reduction; and from the solution an osazone was isolated having the behavior of maltosazone. Emmerling[1] repeated the experiment and obtained a disaccharide that he described as isomaltose. Later Fischer and Armstrong successfully reversed the process of inversion of milk sugar. They allowed a lactase from the kephir yeast to act upon solution containing d-glucose and d-galactose, and isolated a disaccharide which they identified as isolactose. The reason for the appearance of the disaccharide in the form of the isomeric iso-sugar has not been investigated; in any event the question is of qualitative interest solely. From the standpoint of the relations of fermentation to the steroisomerism of sugars, the formation of the iso-disaccharide on the acceleration of the reversed reaction by ferments is of the greatest interest, but it in no way qualifies the validity of the fact that the formation of the disaccharide is accomplished under the influence of a ferment. Armstrong[2] has investigated in detail the products of the reversed action of ferments on the synthesis of disaccharide from maltose. On the basis of the well known work of Fischer and himself on the configuration of the glucosides, Armstrong regards maltose as glucose-*a*-glucoside and isomaltose as glucose-*b*-glucoside. When maltase is allowed to act upon a solution of glucose, as had been previously known, isomaltose is produced. When emulsine is allowed to act upon a similar solution of glucose, maltose is formed. The specificity in the relations of the two ferments to the two forms of glucoside is therefore maintained in the syntheses. When the synthesis was accelerated by the presence of hydrochloric acid, both maltose and isomaltose were determined to be present. Fischer and Armstrong were able further to show that lactase could condense two molecules of d-glucose to a biohexose of undetermined nature. Wroblowski has reported the synthesis of cane sugar from the component hexoses under the influence of invertase. Finally the panereatic amylase has been

shown by Acree and Hinkins to be capable of accelerating the combination of acetic acid and d-glucose to the synthetic triacetyl-glucose. The velocity of these various accelerations of the reversed reactions is very slow; weeks are required to synthesize an amount of the disaccharide that can be hydrolyzed within a few moments. Since these reversions are naturally supposed to occur in nature, it is evident that the conditions in vivo possess certain relations that are lacking in the experiments in vitro.

Relations of total concentration to progress of reaction. The work of Henri has illustrated explicitly what had been approximately indicated in the earlier work, that the relation between the concentration of the substrate and the velocity of the transformation holds only for dilute solutions. The most favorable concentrations are from 0.1 to 0.5 normal solution. This is in marked contrast to the relations in the inversions by acids, which obey the law under much wider limits. For the acid hydrolysis, however, the concentration of substrate may be reached at which the law is no longer obeyed. We have here therefore a quantitative, not a qualitative difference; the operations of the enzymic acceleration of the inversion follow the law within narrower limits of variations in the substrate than in the case of the acid acceleration.

Influence of temperature. In a number of careful investigations the activity of invertase has been shown to increase up to 35° in a quite strict conformity to the law for the increase of reactions for increases in temperature, but from 35° to 55° the increment is disproportionately greater, and above 55° the power of inversion is rapidly destroyed with increasing temperature. There are wide variations for different preparations, and this is equally true for maltase, whose optimum temperature seems to be in general some 15° lower than for invertase. The optimum temperature does not seem to be the same for systems of high and low concentrations of substrate and ferment. Invertase when dry is very resistant to heat, and may be heated to over 100°; maltase is less resistant. When dried both ferments preserve their activity over a long time. Light, in the presence of free oxygen, is destructive to invertase; otherwise not.

For both invertase and maltase a faintly acid reaction seems

advantageous, although at a neutral reaction fermentation is still very active. Alkalies are harmful in the highest dilutions. The susceptibility to these influences, however, varies widely with different preparations; some do best in a 1/300 normal acid, others in one ten times more diluted. The other conditions, especially temperature and concentration of the substrate, influence the effects of the reaction of the medium. Acids act as zymoexciters, not as accessory catalysors. Other substances act as zymoexciters: the chlorides of ammonium, sodium, and potassium, though not to marked extent. The salts of the heavy metals, cyanogen compounds, arsenic, calcium chloride, and alcohol act as negative catalysors, though the necessary minimal quantity varies widely with different preparations. Chloroform and toluol in moderate quantity are of no effect. Fluorescent bodies, that are known to be strong oxydizing agents in the presence of light (Straub, Edlefsen), and that act energetically on bacteria (Jodblauer and Tappeiner), and are also haemolytic (Sacharoff and Sachs), are active depressors to the action of invertase (Emmerling),[2] as they are apparently to all ferments, as well as to many toxines. A solution of eosin of 1:1000000 will depress very materially the action of invertase. Incidentally remarked, these are the only substances known that in traces depress fermentations like the so-called poisons of reactions described by Young, Silow, and others.

Invertase when purified to the highest degree yet reported (Osborne, probably not very pure) is a white powder that is but slightly soluble in water, forming a thick viscid suspension that resists filtration, quite in contrast to the behavior of amylase. Unless invertase be contaminated with some vegetable mucus or gum, it is certainly the most colloidal of the known ferments. Nevertheless the active principle will diffuse. It cannot be gotten ash-free. It contains phosphorus in organic combination, and on being boiled with acids it will reduce Fehling's solution, from which the presence of a carbohydrate moiety is assured. It does not give the biuret test and is very resistant to proteolysis—qualities not at all in harmony with the tentative classification as a glyco-protein that would be suggested by its other characteristics. According to Hopner, invertase is not a

protein, and is entirely resistant to trypic digestion. For maltase and lactase we possess still less data upon their chemical qualities.

EMULSINE.

The fermentation of amygadeline with emulsine was described by Robiquet and Boutron. The ferment is found in almonds, in laurel leaves, in many plants, in a large number of fungi, especially the parasitic fungi of trees, and in some yeasts like aspergillus niger and penicillium glaucum (Herissey). So far as known, emulsine is not found in the animal economy; the alleged presence in the digestive juices is probably due to the presence of bacteria, many of which produce emulsine.

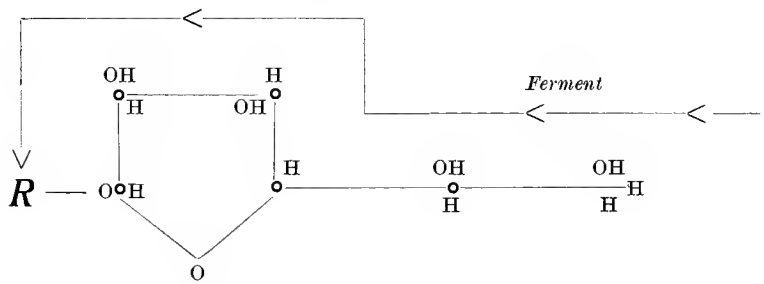
Emulsine is able to ferment a large number of glucosides, and in the inversion these aether-like bodies are split into d-glucose and the other component.

$$C_{13}H_{18}O_7 + H_2O = C_6H_{12}O_6 + C_7H_8O_2.$$
Salicin + water = d-glucose + saligenin.

Emulsine is able to hydrolyze salicine, amygdaline, arbutine, helecine, asculine, phloridzin, coniferin, gaultherin, and daphnin. As a matter of fact emulsine does not ferment amygdaline at all, but only the mandel-nitril glucoside, which is produced by the cleavage of the amygdaline under the influence of maltase. The natural glucosides all correspond to the *b*-glucosides (Pottevin). The number of glucosides that may be fermented by this one ferment has always attracted attention, and these facts have been quoted as illustrating a lack of ferment specificity. In truth, the inference is unwarranted. We are dealing with sugar fermentations; these cleavages in reality represent inversions, and it would be quite natural to believe that it is the sugar moiety in the molecule of glucoside that determines the fermentability. Now in all the glucosides that may be fermented by emulsine but one sugar is contained, d-glucose. Emulsine is not able to ferment myrosin, xantorhammin, rubian, or senalbine. Two of these contain no d-glucose, and the two that do contain it contain not one but two other molecules, though in this they are precisely comparable to amygdaline, which emulsine does not ferment directly. It must not be understood that emulsine is stated to be

able to split all such glucosides as contain d-glucose; but when all the glucosides fermentable by emulsine contain this hexose, the behavior of this ferment may at least not be urged as a direct argument against the specificity of its action. This view of the matter is confirmed by the observation of Fischer that an inversion ferment is able to ferment amygdaline; the pancreatic juice is also known to be able to ferment amygdaline. This inversion ferment is in all probability maltase. That maltase is not able to ferment other natural glucosides might be due to the fact that they contain but one molecule of glucose in combination with the other component, while amygdaline contains two molecules, and these two molecules are combined in a manner analogous to that observed in maltose and in the *a*-methyl maltosides and glucosides. Emulsine for its part is also able to accelerate the hydrolysis of the synthetic methyl-glucosides, but only those of the *b* series. This all is in harmony with the fact that invertase, so far as known, acts only on those disaccharides as contain d-laevulose, and ferments also only those synthetic fructo-glucosides of the *a* series.

Armstrong[5] has recently attempted to define the point of attack in the hydrolysis of glucosides. The configuration of glucosides is assumed to correspond in general to the scheme:



In the act of hydrolysis, the radicle *R* (the aromatic, aldehyde or other component of the glucoside) is replaced by an *H;* in the case of acids, an *H* is first attached to the adjacent O, while in the case of ferments, the attachment may occur at any point along the chain of carbons. Analogous considerations are applied to the galactosides.

Kinetics of the fermentation. Tammann first studied the fermentation of emulsine from the kinetic point of view, employing salecin and amygdaline. He found that the progress of the reaction did not conform well to the regular law for a monomolecular reaction; the march was slower than corresponded to the logarythmic curve. In general terms the velocity was only roughly proportional to the concentration of the substrate, though for concentrated solutions the variations were wide. The reaction was always incomplete. He failed, however, to bring about a direct reversion by the action of emulsine upon a solution of the products. The addition of products depressed the reaction, and the entire cessation of the fermentation could be brought about by the early addition of products. The addition of substrate to a quiescent mixture resulted in the reëstablishment of the hydrolysis, and this same result could be secured by the withdrawal of a portion of the products, by an increase in temperature, by dilution, and by the addition of additional ferment. He found that solutions of emulsine undergo hydrolysis, both in simple solution and in the fermentation test. Tammann deserves the credit of having first approached the question of the kinetics of ferment action with a full appreciation of the problem. He attempted to represent the progress of such a reaction in an equation based upon the idea that the transformation was in the unit of time proportional to the active mass of the substrate and of the non-inactivated (or better undestroyed) ferment. Thus $-\frac{dx}{dt} = C(A-x)(F-y)$, in which A and F are respectively the original amounts of substrate and ferment, x and y the amounts of converted substrate and destroyed ferment in the time t, C the constant of velocity. The results of calculations according to this equation do not give good results, and the reason for this is that while the destruction of the ferment is a simple monomolecular reaction running alongside the main reaction, the ferment combines with the components of the main reaction, and during these combinations it is protected from hydrolysis.

Henri[6] has lately worked over the action of emulsine upon salacine. He confirmed the statements of Tammann that the

reaction proceeds slower than represented by the simple logarythmal curve, that the proportionality between substrate concentration and transformation is not exact, that the products depress the reaction, and that the velocity was in a general way proportional to the quantity of ferment. He, however, found that the ferment, in diluted systems at low temperature where the fermentation did not last over seven hours, was preserved intact for all practical purposes. He then attempted to apply to this reaction the formula $C = \frac{1}{t} \log \frac{A+x}{A-x}$ that had been obtained empirically for invertase, but since the reaction is slower than corresponding to the logarythmal curve, the results were worse than with the regular equation $C = \frac{1}{t} \log \frac{A}{A-x}$. Henri then applied his theoretic formula as described under invertase. The two applications are different in the sense that applied to invertase m (constant of equilibrium with substrate) is greater than n (constant of equilibrium with products), while in the case of emulsine n is greater than m, corresponding to the facts that in the invertase fermentation the progress of the reaction is more rapid and in the case of the emulsine fermentation slower than the logarythmal curve. He ascertained that $m =$ about 40, and $n = 120$. When the experimental data was calculated according to this equation, the constants were fairly uniform. While Henri ascribes this not fully exact conformation to the not entirely accurate figures for m and n, one cannot resist the feeling that this may have been due to inactivation of the ferment.

Visser has applied his equation to the action of emulsine. For this ferment he has determined of course a different intensity of action than that of invertase. This he found to be: $I = \frac{Const.}{4A^2 - 2A(A-x) - (A-x)^2}$. When a system containing a glucoside and emulsine in the commonly employed concentration is at rest, over 96 per cent. is hydrolyzed; therefore the intensity formula may be inserted, as in the case of invertase, into the simple equation: $-\frac{dx}{dt} C(A-x)$. This gives us: $-\frac{dx}{dt} = C(A-x) \cdot \frac{C^2}{4A^2 - 2A(A-x) - (A-x)^2}$ and this when integrated under the assumption that $x = 0$ when $t = 0$, yields: $2\ C_1 C_2\ t = 8\ A^2\ l \frac{A}{A-x} - (5A + (A-x))x$. The results

are not satisfactory in that they do not yield an agreement in the constants with series in different concentrations of the substrate.

Herzog has also applied his equation to the reaction of the fermentation of glucosides. The results were fairly concordant with the theory. What is rather surprising in the results of Henri, Visser, and Herzog is the concordance in the results despite the fact that emulsine is one of the most sensitive of ferments. While in some instances it is true, as stated by Henri, that the ferment will preserve itself intact through a test of not over seven hours, this is very liable not to be the case; many preparations of emulsine will not yield such regular results. It is quite likely that the intensity (I) of the equations of Henri and Visser include the factor of inactivation, though no such variable is predicated in the development of the equations. Probably the inactivated fraction would be included in that portion supposed to rest in combination with the products.

The *temperature optimum* of emulsine lies about 40–45°. To a certain extent it varies with the concentrations in the system. Increases in temperature are accompanied by such increase in the velocity of transformation as would be expected from the law of such increase.

The *reversion* of the glucosidic fermentation has been accomplished by Emmerling. The reaction for the hydrolysis of amygdaline is as follows:

$$C_{20}H_{27}O_{11}N + 2\,H_2O = C_6H_5.COOH + HCN + 2\,C_6H_{12}O_6.$$

Amygdaline + water = benzaldehyde + HCN + d-glucose.

This reaction has never been reversed, possibly on account of the action of the hydrocyanic acid upon ferments. Fischer has shown that a ferment exists in the yeast that acts to split amygdaline into mandel-nitril glucoside and d-glucose.

$$C_{20}H_{27}O_{11}N + H_2O = C_{14}H_{17}O_6N + C_6H_{12}O_6.$$

This ferment is probably maltase. Emmerling mixed with these substances a maltase derived from yeast and months afterwards isolated amygdaline from the mixture.

Emulsine is not a resistant ferment; it may be easily preserved; it is quite resistant to the action of salts, though mod-

erate amounts of acid or alkali depress it. The optimum reaction is slightly acid. Emulsine has never been purified; it is not possible to separate it from the other ferments that accompany it. It is very resistant to proteolytic digestion. The ferment is soluble in water with but slight opacity and without marked colloidality; it may be filtered through infusorial filters; it gives the common reactions for protein, rotates light to the left, and yields on heating with orcein a deep orange color that reminds one of pentose.

FERMENTATION OF MONOSACCHARIDE. ALCOHOLIC FERMENTATION.

Of the numerous fermentations of primary sugars those affecting the hexoses are best known. And of the fermentations of the hexoses that affecting d-glucose and leading to its cleavage into aethyl alcohol and carbon dioxide is best known, and to this one we will give our entire attention. The scientific study of alcoholic fermentation dates back to the genial prophet of the law of the conservation of energy, Lavoisier. He determined that in this fermentation the sugar is converted into alcohol and carbon dioxide, both bodies containing oxygen. The quantitative relations were determined by Gay-Lussac and Dumas, who wrote the reaction as we write it to-day. It is very interesting, in the light of modern views on the reversibility of ferment action, to observe that Lavoisier was convinced that were it possible to combine aethyl alcohol and carbon dioxide, the product would be glucose. Neither Lavoisier nor Gay-Lussac paid any attention to the fungus skum that is a macroscopic appearance in all alcoholic fermentations.

Some forty years later de Latour and Schwann at about the same time independently described the presence of yeast cells in fermentations, and stated the view that upon the life processes of these vegetable cells depended the conversion of the sugar into alcohol and carbon dioxide. They recognized that inoculation occurred from the air, and considered that the process of fermentation stood in some relation to the nutrition of the plant.

They indulged in little speculation upon the nature of the phenomenon, and though they felt the truth, they did not extend their studies to any great extent, and their publications had little immediate effect. It was Pasteur who developed this theory, proved its truth in the most manifold ways, and made the scientific and industrial world believe in it. Next to Pasteur the biology of fermentation owes most to the work of Hensen and Duclaux.

Prior to the advent of Pasteur upon the scene, the chemists simply satirized the yeast theory; the work of Pasteur could not be thus disposed of, and was bitterly combatted. Possibly in no scientific discussion has the personal adjective been oftener used. Even now the latest facts in the general theory of alcoholic fermentation are often so interpreted and expounded as to constitute a balm to the historical sense. It is now quite the fashion to say that Liebig and Pasteur were both in the right. This is not true; the statement does no credit to either Pasteur or to Liebig, both of whom were too great to desire spurious reputation, and does historical injustice to another man. In this whole controversy there was, upon the part of the adherents at least, too little of the spirit of the great Faraday, who considered a theory as a question put to Nature: if she answered yes, well and good; if she answered no, he sought another path.

Pasteur demonstrated that in fermentations as they occur in nature, germs are always present; when germs are excluded, fermentation does not occur. The work of Schroeder had in its technical details as well as in the results anticipated many of the studies of Pasteur. Mitscherlich had previously shown that fermentation could not extend through a diffusion membrane, and Helmholtz had confirmed this for animal membranes. Years afterwards Dumas in a beautiful experiment showed that when two layers of widely different specific gravities were carefully placed upon each other, a fermentation would not pass from one layer into the other. Pasteur, however, went beyond the demonstration of the causative relation of yeasts to fermentations, as was most natural he developed a theory of fermentation. This theory was in brief that alcoholic fermentation was the result of vegetation without air, and that it represented a compensatory

functionation whereby the yeasts were enabled to live in a medium without oxygen by making the oxygen of the sugar available for utilization. It was, however, promptly shown, especially by Schuetzenberger, that alcoholic fermentation occurs as well in the open atmosphere and even in an atmosphere of oxygen as under anaerobic conditions. The answer of the Pasteur party to this was that at some distant past time the yeast had acquired the activity as a so-to-speak vicarious function, and that once acquired the function continued to be exercised whether the conditions that originally called it into being were present or not. This application of the doctrine of phylogeny to chemical function was too far fetched to exert a prolonged conviction. Later in his life Pasteur modified his views to some extent, and even attempted, though without the energy born of conviction, the isolation of a ferment from the bodies of the yeast cells; but he never yielded the general idea that fermentation constitutes a so-so-to-speak vicarious method of respiration,—every yeast cell its own oxygen generator. As true as was the theory of Pasteur upon the biological origin of natural fermentations, equally improbable was his theory of the chemical nature of that fermentation. Some modern botanists, like Stoklassa, associate the function of alcoholic fermentation with the intracellular respiration of plants, though not in the sense employed by Pasteur.

The theory of Liebig sounds to us to-day strangely mystical. According to him, the fermentation of a sugar was a process which the sugar contracted, so to speak, from contact with some other body undergoing the same or some similar disintegration. The cause of fermentation, he thought, lay in the tendency possessed by substances in process of chemical action to convey this same chemical action to another body in proximity to it, or at least to make the neighboring body susceptible to it. The nature of the transmission he conceived to be a sort of molecular vibration. When a body in process of decomposition lay beside another body, the molecular vibrations were transferred to the second body with the result that it thereupon underwent the same decomposition. Ferments were thus defined as bodies in process of disintegration, and the alcoholic fermentation was the result of the disintegration communicated to the sugar molecule.

The conception was applied to all the animal ferments by Naegeli. When Liebig finally realized the force of the experiments of Pasteur that fermentations never occur in the absence of germs, he shifted his ground by locating the decomposing body in the yeast cell. It is an error to state that Liebig simply fought for a chemical interpretation of fermentation as against a vitalistic interpretation. Liebig contended as well for a particular chemical interpretation of fermentation, after as much as before his admission of the etiological relations of the yeast germ.

Very recently Barendrecht has proposed a hypothesis for fermentation that recalls vividly the hypothesis of Liebig. Barendrecht considers the catalytic activity of a ferment to lie in emanations that proceed from it and act upon surrounding molecules. This idea is obviously a far-fetched application of the earlier, now admittedly erroneous interpretation of the phenomenon of so-called induced radio-activity. The emanations are considered to be absorbed by the substrate and the products of a reaction, and upon this an equation is developed. Now it cannot of course be denied that the acceleration of a reaction through the medium of intermediary reactions may be due to the action of emanations, electronic or otherwise, but it is the wildest speculation. The sympathetic reference to the n-rays indicates clearly that the author is emanationally inclined. For us it is, in the present state of our knowledge, sufficient to dismiss the subject with the remark that the sheep of Badenrecht is only the wolf of Liebig dressed up in modern emanation wool.

At the very height of this controversy, 1858, what we today regard as the true theory of fermentation was enunciated by Moritz Traube. It was not a casual statement, but a perfectly conscious and deliberated promulgation of a definite theory. Traube was a resourceful theoretical as well as a brilliant qualitative experimental chemist. He had worked much upon the qualitative nature of reactions, and especially upon catalytic reactions. He had a fine sense of perspective, and was content to state his theory without becoming engaged in the contemporaneous polemic and to await the future experimental development of that theory. He formulated the view that there was in the body of the yeast cell a product of its metabolism, though in

no concrete way defined, a substance which once existent reacted with sugar with the production of alcohol and carbon dioxide. And secondly this reaction between the hypothetical substance and the sugar was a reaction entirely independent of the parent cell, and in its chemical nature and relations similar and related to the catalytic reactions. Traube knew that the catalytic decompositions occurred slowly in the absence of the catalysor. The definite separation of the reaction de novo and the accelerated reaction is, however, due largely to Ostwald, who has through his work secured the general application in chemical reasoning of this distinction. The work of the last forty years has resulted in the verification of the theory of Traube, though he did not live to witness the isolation of zymase. The work of Traube was quickly recognized by Berthelot and Claude Bernard in France, and by Scloenbein, Hoppe-Seyler, and von Manassein in Germany. Bernard attempted the isolation of the ferment, as did Naegeli, Loew, Mayer, von Manassein, and even Pasteur, all with negative results. In 1897 E. Buchner[1] isolated the active substance in a cell-free form. Miquel had previously isolated the ferment urase from the micrococcus urea.

When we speak of the isolation of the alcoholic ferment from the yeast cells, we mean as yet only isolation in the same rough sense that the term was used in connection with amylase, and invertase. What is obtained is a fluid extract of the yeast cells, and this contains among other inorganic and organic constituents the active ferment. This extract has been purified to some extent, but up to the present zymase has not been prepared in as pure condition as many of the other ferments. The method of preparation of the extract is simple. The yeast (zymase is found in many fungi) is first washed until all the particles of light gravity have been removed and the wash water flows clear; this is best done by a combination of straining and decantation. It is then dried under pressure, about fifty atmospheres being necessary. The resultant powder is still two-thirds water. This powder is then mixed with an equal weight of quartz sand of finest grain and about one-fourth weight of infusorial earth that has been washed, ground, and calcined. This mixture is then ground to a fine powder, as determined by microscopic appearances.

Macroscopically the powder assumes a plastic dough-like consistency, due to the absorption of the cellular juices by the infusorial earth. When the microscopic examination indicates that nearly all the cells have been crushed, the mass is placed in a heavy canvas cloth and submitted in a hydraulic press to a pressure of some 300 atmospheres. In all probability the new method of MacFadyean and Rowland for grinding germs at the temperature of liquid air would yield better results. Not only is the trituration of the cells accomplished best at this low temperature on account of the increased brittleness of the tissue, but the mass would not assume such a plastic consistency on account of the freezing of the water; disintegration of the juice also would be inhibited. The yield of juice is about three-fourths of the water content of the yeast; a second extraction after regrinding will yield a little more. The juice should be filtered, and kept at very low temperature.

A more recent method developed in the laboratory of the pharmacologist, H. H. Meyer, yields much better results. The yeast cells are simply placed in a vacuum and the space filled with ether vapor. Fuid soon begins to pass from the cells; this fluid is rich in zymase, and may be recovered by simple filtration with pressure. Just how the procedure operates is not clear. As Loeb has suggested, one might infer the process to be autolytic. What one cannot in addition understand is the process of diffusion, since it has been shown that in natural alcoholic fermentation the reaction occurs within the cells, the sugar diffuses in and the products out, but the ferment does not and apparently cannot diffuse out.

The yeast juice is of a yellowish color, somewhat opalescent (due in part to colloidal infusorial earth); a slightly viscid fluid, practically neutral, with the odor and taste of yeast. After filtration through an infusorial filter, the fluid is as clear as water, and does not display more than a trace of opacity when illuminated with oblique light. Filtration through an infusorial filter usually carries with it loss in enzymic power, though it varies much with different preparations and is least when done at the lowest feasible temperature. The loss during such a filtration is highest at the beginning; if one collects only the last half of

a large filtration, the activity will be found not seriously reduced. Porcelain filters work more slowly, and do not seem to give better results. Such filtration is necessary to exclude isolated yeast cells, bacteria, and also the colloidal infusorial earth.

The yeast juice will not dyalize through a parchment membrane with measurable rapidity. This observation is in accord with the experience that it has never been possible to detect fermentative action in the fluid in which yeast cells are suspended. Groblewski, for example, filtered through a sand filter a solution of sugar containing yeast and presenting active fermentation, but the filtrate was entirely inactive. This practical inability of the ferment to dyalize is interpreted to indicate that in nature the fermentation occurs within the cell, the sugar dyalizing in and the alcohol and carbon dioxide diffusing outwards.

The extract is stated not to polarize light. It has a specific gravity of 1030 to 1050, contains from 11 to 14 per cent. of solids, from 1.5 to 2 per cent of ash, one-fifth per cent. of phosphorus, and from 13 to 15 per cent. of nitrogen; that is, the solids contain some 13 to 16 per cent of nitrogen. The extract is very rich in protein, gives the biuret, Millon, and Xanthoproteic reactions, and contains more or less glycogen. The proteins have a low coagulation point; even at 45° the first coagulation is noted, while complete coagulation occurs at a little over 60°. The addition of strong acids or alkali will precipitate heavily in the cold. As indicated by the rather high content in phosphorus, the protein consists largely of neucleo-proteid, and this is confirmed by the appearance of purin bases on digestion. On autodigestion the usual amido-acids noted in protein digestions in general are produced, and also purin bases.

The yeast extract possesses rather marked powers of reduction. Thus nitrites are reduced with the production of gaseous nitrogen; hyposulphites are reduced to hydrogen sulphide; alkaline copper and silver solutions are promptly reduced in the cold; sulphur is converted into H_2S, and also into mercapten. These reductions are not dependent upon the zymase; they depend upon bodies that are soluble in alcohol, and remain after the ferment is destroyed. The ability to reduce methylen blue, however, disappears with the destruction of the ferment.

The extract is very unstable from the standpoint of the zymase. This liability seems to be due to autodigestion; that is, to the digestion of the specific ferment by the active proteolytic ferment present in the extract. A low temperature retards this digestion. The destruction of the zymase in the extracts Buchner found entirely independent of atmospheric oxygen. That the digestion is the cause of the destruction of the zymase is rendered very probable by the fact that the destruction of zymase and the diminution in the coagulable protein diminish pari passu. When dessicated at low temperature in vacuo the powder is very stable, can be conserved a long time without serious loss of specific activity, and bears heating up to 100°.

This extract contains a number of ferments: *a.* the alcoholic ferment, zymase; *b.* the proteolytic ferment, yeast-endotryptase a very active and destructive ferment; *c.* invertase; *d.* maltase; *e.* some form of amylase since glycogen is hydrolyzed, and even starch, though very slowly; *f.* substances that will react with hydrogen peroxide, and the reducing bodies already mentioned, though what relations these may bear to the enumerated ferments are not known. Of the sixteen isomeric hexoses (twelve of which have been prepared) the alcoholic ferment in zymase is able to accelerate the fermentation of but four: d-glucose, d-mannose, d-fructose, and d-galactose, the last with difficulty. The extract may be concentrated by careful freezing; the last fluid is very rich in ferments. Many attempts have been made to free the zymase from the other bodies. The least unsuccessful methods have been by precipitation with alcohol-aether and with acetone. Through these procedures some of the alcoholic ferment is lost, and much of the other material retained. The precipitated powders keep well, but it is doubtful if the procedures are of much value from any other point of view. The multiplicity of ferments in the extract of one yeast illustrates how worthless have been the discussions as to whether the fermentative activities in crude amylases, invertases, etc., were due to one or more ferments, and whether all the invertases were alike, etc.

The yeast extract will invert saccharose and maltose rapidly, and will ferment alcoholically d-glucose and d-laevulose with rapidity. Raffinose is slowly fermented, glycogen and d-galac-

tose very slowly, while starch is hydrolyzed with the greatest difficulty. Lactose is not inverted at all. These activities correspond in general with those noted for fermentation with yeast, except that yeast cannot ferment glycogen at all; this is supposed to be due to inability of diffusion upon the part of the glycogen.

Zymase appears to act best in a reaction of faint alkalinity. If the fermentation be done in a closed chamber, it is apparent that when the fluid becomes saturated with carbon dioxide no alkaline reaction can be maintained, and the conditions would be as they are in the blood, practical neutrality. All acids seem to depress; partly by accelerating the digestion by the endotryptase, and partly by true inactivation. Neutral salts possess either no action, or they depress the fermentation. Chloroform, toluol, thymol, and glycerine have little action. Weak solutions of arsenite do not inhibit; strong solutions do so, as do all cyanogen compounds. On account of the complicated conditions, referable especially to the endotryptase, the data upon the influence of extraneous substances are difficult to value. The alkali phosphates (the PO_4 is the active factor) are group zymo-exciters to zymase, as is curiously enough also, lecithin. When yeast is allowed to lie for some time in a solution of cane sugar containing asparagin, the zymase from it will be found very active. (Buchner.)

Harden[1] has investigated in detail the zymoexcitor for alcoholic fermentation. While not denying the activity of phosphates, he points out that an organic substance in the yeast extract is much more potent. If a yeast extract be allowed to undergo autodigestion, then boiled and filtered, this fluid is most active as a zymoexciter. The active substance may be precipitated by alcohol at 75 per cent. concentration, is thermo-stabile in solution, but is destroyed on being ashed. The substance diffuses easily; it is therefore crystalloid. Harden goes so far as to suggest that zymase alone cannot accelerate the reaction of fermentation, but the experimental evidence is not sufficient to support this. Certain it is, however, that the zymoexciter exerts a most marked stimulation to the fermentative action of the zymase. The zymoexciter alone is not an alcoholic ferment.

Buchner and Albertoni believe that the substance in yeast extract described by Harden and Young as a co-ferment for zymase

is phosphoric acid. This accelerates markedly the fermentation by zymase. Organically combined acid, as in lecethin, had the same action.

Harden and Young have shown that when a phosphate is added to a fermenting system, it is on the completion of the reaction not recoverable by precipitation with magnesia mixture or silver nitrate.

Stoklasa has recently published the statement, accompanied by the experimental data, that he has been able to isolate zymase from different plants; and also from mammalian tissues a cell-free extract that has the power of fermenting sugar. The experiments are convincing (assuming that the bacteria have been effectively excluded, which has been denied by Mazé and others), but require confirmation. Should the observation prove to be correct, the fact will constitute one of fundamental physiological importance, since the modus of sugar combustion will be thereby elucidated. The presence of alcohol in mammalian tissues was long ago determined by Hoppe-Seyler and Akari, Rajewski and Béchamp. Maignon has recently gone over the ground anew, and found alcohol invariably in muscle, even after death.

The reaction of alcoholic fermentation. The auto-fermentation of d-glucose has been demonstrated by Duclaux. He exposed sterile solutions of the sugar, of a faint alkaline reaction, to sunlight, and after a time was able to demonstrate the presence of aethyl alcohol. Colloidal platinum acts also as an accelerator to the auto-reaction. The simple reaction is $C_6H_{12}O_6 = 2\ C_2H_6O + 2\ CO_2$. The equation obviously represents but the initial and the final stages. It is not possible to write a rational equation based upon the sterio-isomeric formula of d-glucose that will illustrate the direct cleavage of the sugar into alcohol and carbon dioxide. Intermediary reactions must be assumed, and in all probability these demand the temporary addition of other elements. Water is quite certainly engaged in the reaction of fermentation. Baeyer proposed the first explanation as early as 1870. His conception was that water was added to the molecule of sugar in what we would now call ionic form; following this the hydroxyl groups underwent a rearrangement whereby they were accumulated at two points, with the consequent reduction

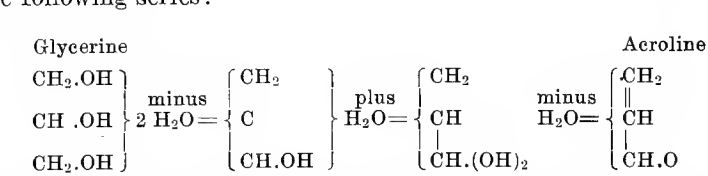
of two other groups; the carbon chain was then broken at the points of the accumulation of oxygen. The theory holds for simpler compounds as well, and the processes are quite identical with those frequently observed in connection with the action of those substances that at high temperatures effect the withdrawal of water from an organic molecule.

When propyl alcohol is heated with sulphuric acid it is transformed into propylen, which in its turn again binds water in a different manner to form isolpropyl alcohol.

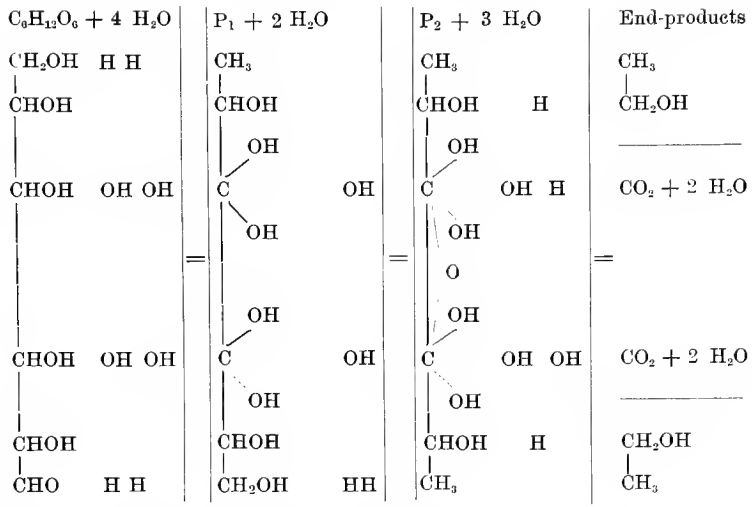
Propyl alcohol minus water = propylen.
$CH_3.CH_2.CH_2OH - H_2O = CH_3.CH.CH_2.$
Propylen plus water = isopropyl alcohol.
$CH_3.CH.CH_2. + H_2O = CH_3.CH(OH).CH_3.$

The process consists obviously in the transfer of one hydroxyl group from one atom of carbon to another. Another frequent illustration of such a transfer of hydroxyl groups from one carbon to another is seen in the rule that in the use of substances that abstract water, the group CH(OH).CH(OH). passes into $CH_2.CO$, the reaction passing through the intermediary unsaturated alcohol CH:C(OH):. In a similar manner oxalic acid is split into formic acid and carbon dioxide, etc. That high temperature is not necessary has been shown by Wohl and Oesterlin, who have shown that d-tartaric acid (COOH.CH(OH).CH(OH). COOH.) through the successive actions of aectyl chloride and pyridine may be converted at low temperature into oxalacetic acid ($COOH.CO.CH_2.COOH$). The reactions whereby hexoses may be converted into each other by the action of alkali has also beeu explained in an analogous manner by Lobry de Brun and Van Erenstein.

The formation of acroline from glycerine is represented by the following series:

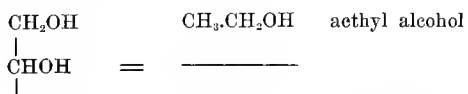


Applied to the fermentation of d-glucose we have the following stages.



This equation illustrates that it is possible to write the reaction for the fermentation of d-glucose with but the single assumption of the successive addition and subtraction of water, with the translocation of hydroxyl groups. The last intermediary stage represents the anhydride of lactic acid. The reaction, *i.e.*, the production of alcohol, has never been accomplished for d-glucose by the simple action of heat alone; thus far the yield has been lactic acid and not aethyl alcohol. Quite similar conceptions have been advanced by Rayman, Mayer, Wagner, and by Wohl.

Harden[2] has recently studied the fermentation of d-glucose by the Bac. coli communis. He found that alcohol, lactic and acetic acids were regularly produced, and believed that the acids are produced from the sugar directly and not from the alcohol by oxidative fermentation. He found that the yield in alcohol was least in the fermentation of d-glucose, greater in mannitc, and greatest of all in glycerine. This fact he brought in relation to the number of $CH_2OH.CHOH$ groups, and postulated the view that the aethyl alcohol was derived only from this group. Thus for glycerine we have



Mannite yields for each molecule one of aethyl alcohol, as is seen in the equation, written for two molecules.

Mannite.

$$\begin{array}{ll} CH_2OH & \\ CHOH \quad CH_2OH & = CH_3.CH_2OH. + CO_2 + H_2 \\ \hline CHOH \quad CHOH & \\ CHOH \quad CHOH & = \text{lactic acid, ect.} \\ CHOH \quad CHOH & \\ \hline CH_2OH \quad CHOH & \\ \qquad\quad CH_2OH & = CH_3.CH_2OH. + CO_2 + H_2 \end{array}$$

In the case of d-glucose, on the contrary, one molecule of aethyl alcohol is produced for each two molecules of sugar.

d-glucose.

$$\begin{array}{ll} CH_2OH & \\ CHOH \quad CH_2OH & = CH_3.CH_2OH. + CO_2 + H_2 \\ \hline CHOH \quad CHOH & \\ CHOH \quad CHOH & \\ CHOH \quad CHOH & = \text{lactic acid, ect.} \\ \hline CHO \quad CHOH & \\ \qquad\quad CHO & + H_2O = CH_3.COOH + CO_2 + H_2 \end{array}$$

Now this closely resembles the Baeyer scheme. Granted for the sake of argument that the equation represents a single process, it is apparent that it will not apply to the alcoholic fermentation of d-glucose by zymase because the entire mass of sugar appears as alcohol and carbon dioxide. Under such circumstances alcohol must come from the numerous CHOH groups, and the Baeyer theory represents the simplest conception of securing aethyl alcohol from these groupings. Indeed it is apparent that the intermediary reactions in the Baeyer equation can be written almost directly into the Harden equation.

This theory of course does not explain the reaction of fermentation in the desired concrete sense. But it does place the reaction upon the same plane as many other reactions of organic

compounds, and thus the problem of alcoholic fermentation becomes simply one of the problems connected with reactions in the asymetrical carbon compounds. That the Baeyer theory fits well into the doctrine of catalytic acceleration will become obvious when we consider the catalyses from the point of view of successive intermediary reactions.

From the standpoint of organic chemical behavior the Baeyer theory has one doubtful step, the reduction of the aldehyde group. Buchner and Meisenheimer have recently worked upon the matter experimentally, and have developed a different formulation of the equation that not only avoids the supposition of a reduction of the aldehyde group, but also indicates the formation of alcohol from the CHOH group.

Glucose + 4 H_2O.		P_1 + H_2O.		Lactic-acid.	2 H_2O.	Products
	OH					
CHO	OH	COOH		COOH	OH	CO_2
|		|		|	|	
CHOH		CHOH		CHOH	H	CH_2.OH
|	H	|		|		|
CHOH		CH_2	H	CH_3.		CH_3.
|	H	|				
|	OH	|				
CHOH		CO	OH	COOH	OH	CO_2
|	OH	|		|	|	
CHOH		CHOH		CHOH	H	CH_2.OH
|	H	|		|		|
CH_2OH		CH_2		CH_3.		CH_3.
	H					

They were able to detect the transient presence of lactic acid in the system, and they have therefore revived the older theory that possibly two ferments might be concerned, one transforming the sugar into lactic acid, the other converting the lactic acid into alcohol and carbon dioxide. For this suggestion they were able to produce no experimental evidence, and there can be no doubt that the general tendency expressed in such a suggestion is unsound. If we are to assume a different ferment or catalysor for each successive intermediary stage in the reactions concerned, we shall soon be in the darkness of hopeless confusion. The tendency to invoke new powers when difficulties present themselves is one unfortunately deeply grounded in biological studies with chemical and physical aspects. When the data are not sufficient to permit us to decide a question upon the scope of the known

factors, that same data cannot be employed to justify the invocation of new factors. The mere fact that we cannot understand how one ferment could ferment d-glucose to lactic acid and also ferment lactic acid to alcohol and carbon dioxide is no reason in our present state of knowledge for inferring that two ferments must be concerned. It cannot, on the other hand, be denied that two ferments might be concerned.

In a more recent communication Buchner and Meisenheimer infer methyl glyoxal to be the intermediary stage between sugar and lactic acid. They were able to obtain lactic acid as well as alcohol as the result of the action of alkali on sugar. They are definitely convinced of the plurality of the zymase, and speak of zymase and lactacidase. Stoklasa in a recent paper gives expression to the same assumption of the dual nature of zymase. The zymase is held to act to the stage of lactic acid, the lactacidase following this to the stage of alcohol.

Another scheme for the reactions has been given by Erlenmeyer. $CH_2OH — CHOH — CHOH — CHOH — CHOH —CH O$. This undergoes intramolecular rearrangement into $CH_3 — CO — CHOH — CH_2 — CO — CHO$. This is an aldo condensation product of two molecules of pyro-tartaric aldehyde, $CH_3 — CO — CHO$. This substance passes easily into lactic acid, $CH_3 — CHOH — COOH$ by the addition of water. The lactic acid is then converted into alcohol as in the other schemes.

The experiences with zymase have thrown a very interesting light upon the qualitative reaction in an alcoholic fermentation. From the earliest days, indeed since very shortly after Gay-Lussac studied the reaction, the fermentation of a primary sugar has been supposed to yield four products—aethyl alcohol, carbon dioxide, succinic acid, and glycerine. When it came to the consideration of the quantitative yield, the complexity became apparent. In the attempt to account for the products directly, the chemists tried to so write the reaction as to account for all the experimental facts. Now the attempt to derive the four products in a quantitative manner from the molecule of sugar was enough to exhaust the patience even of a chemist of the analytical type, and numerous different equations were the result. Pasteur, for example, wrote the reaction : 98 glucose + 60 water = 24 suc-

cinic acid + 144 glycerine + 60 carbon dioxide, and according to him this reaction occurred in 4 or 5 per cent. of the substrate, while the rest was fermented entirely to alcohol and carbon dioxide. That these bodies might have nothing to do with the alcoholic fermentation per se, that they might be secondary products, was suggested, but rejected. From the biological point of view this was natural; from the chemical point of view it was illogical. We know now, from the studies on zymase, that glycerine and succinic acid are not products of alcoholic fermentation at all, and do not occur in a pure zymase fermentation. These were apparently metabolic products of the yeast cells, and had nothing more to do with the fermentation than the excretion of urobilin has to do with the carbon dioxide output of the respiration. The matter is of importance in connection with the question of the specificity of ferment action. The glycerine is in all probability derived from the cleavage of the fats of the yeast germs. That it does not come from the sugar has been made very probable by some ingenious experiments of Rodriques Carrando.

Kinetics of the reaction of alcoholic fermentation. The earliest attempts at the study of the kinetics of alcoholic fermentation were made by Dumas, who observed that the period of fermentation with a constant initial inoculation of yeast was in general proportional to the amount of sugar. Cochin next measured the reaction by estimating the carbon dioxide. His figures were quite irregular. Brown did some further tests, and reduced his figures to a curve, which fell away from the line of the logarythmal curve. If brewers made regular observations of temperature, alcohol content, and duration of the fermentation, this data, repeated in numerous instances, together with the known mass of sugar and yeast employed, would in the course of years probably lead to invaluable results, more valuable surely than are to be obtained in a few studies with zymase fermentation.

The Buchners did not carry out detailed experiments designed to determine the quantitative relations. They reached the general conclusion that the yield in products in a unit of time was proportional to the concentration of the substrate. At low concentrations numerous fluctuations were determined. Macfadyen, Morris, and Rowland did not obtain the same result, but

a repetition of the experiments by the Buchners confirmed their earlier findings. The same rule was noted for the relation of velocity to concentration of ferment; this was usually found to be roughly proportional. At very high dilutions of ferment the results were often very conflicting. This was in all probability due to the varying stability of the ferment, to the glycogen-content, and to the fact that the digestion by the endotryptase was accelerated in dilute solutions. The interesting observation was then made that at these high dilutions the integrity of the ferment could be maintained by the addition of egg albumen. The protecting action of this protein the Buchners referred to the colloidal qualities of the egg albumin, and they brought the phenomenon in parallel with the physical observation of the supporting action that colloids of the same electrical sign display in mixed suspensions. This explanation is very probable; but it is also possible that the egg albumin acts by binding the endotryptase. Although specific studies were not published illustrating the stability of the ferment during the reaction of fermentation, the general impression derived from the perusal of the Buchner work is that the ferment was not well preserved during the course of a fermentation.

Herzog next studied the fermentation of glucose. He employed a powdered yeast that had been killed by acetone. Such an aceton-yeast preserves its activity for a long time, but could not be expected to yield as good results as the extract. Nevertheless the results were quite regular, since he employed but one preparation, and made sure that it was a homogeneous mixture. He found that different preparations displayed widely varying degrees of glycogen content and fermentative activity, and that all preparations were rapidly inactivated in solution. This inactivation he determined was due to the destruction of the ferment by autodigestion; indeed he went so far as to state that fermentation may not continue after the completion of autodigestion. Antiseptics were not employed, as the author was convinced that but few bacteria could develop in the presence of the yeast powder, to which he ascribed an antiseptic action. He employed normal solutions of d-glucose and d-laevulose; the reaction was measured by collection of carbon dioxide. The tem-

perature was from 24° to 28°; no attempt was made to maintain a constancy in reaction. Fairly constant results were obtained with from 1 to 2 per cent. concentrations of the ferment in the practically normal solution of sugar; more diluted quantities of ferment gave very irregular reactions, especially at the higher temperatures. The results of a dozen series seemed to indicate that the reaction follows the ordinary logarythmal curve; the values were often irregular, and a tendency to an increase in the value of the constants with the progress of the reaction was definitely apparent in many of the series. When calculated according to the Henri empiric equation for an auto-catalytic reaction, the constants were less satisfactory; not only were the fluctuations greater, but there was a tendency to a reduction in the value of the constants, and these reductions were more pronounced than were the increases in the case of the constants calculated according to the regular equation. Although Herzog did not enter into the question of an interpretation of these results, it seems probable that a progressive inactivation of the ferment (due to destruction and not to influence of products upon transformation) was opposing an increase by auto-catalysis, and the results represent a balance between these factors as applied to the march of a simple monomolecular reaction. Herzog found that the initial rate of transformation with a constant ferment was in general proportional to the concentration of the substrate. He found also that the rate of fermentation with constant concentration of substrate was proportional to the square of the quantity of ferment, and that the increased velocity on increase of temperature was in accordance with the van 't Hoff-Arrhenius equation for such increases in general reactions. Herzog was not satisfied with the experiments, and he himself stated only what every reader must feel on studying the conditions and results of the experiments. It will be necessary to secure a greater purity in the ferment before definite results can be obtained; the march of the fermentation of the sugar seems entirely too dependent upon the march of the digestion of the ferment by the endotryptase and upon the glycogen in the yeast. Herzog's results were indeed much better than will be often obtained by any one who will repeat the experiments, as I can testify. With no

ferment with which I have worked have slight deviations in the conditions exerted so bizarre an influence upon some one of the variables concerned. For myself, I have become convinced that to work successfully with a yeast powder (and probably with an expressed zymase) one must have cultivated the yeast for several generations and fixed its activity, or, to use a common expression with bacteriologists, heightened and standardized its virulence. Certainly the commercial Sachromyces Cerevisea of this city is worthless for the preparation of a yeast powder: the glycogen-content is high, the proteolytic ferment active, the zymase weak. This experience is of course not new. Several of the chemists and bacteriologists who attempted to repeat the original experiments of Buchner failed entirely, and even the discoverers have at times failed, especially with yeast taken from the later stages of a fermentation. The experience of brewers has long been that even under the most favorable conditions a certain race of yeast will become weakened, and Hayduck has described a method of "regenerating" such a yeast by cultivating it upon a medium rich in sugar but poor in nitrogen. Green has suggested that the secretion of zymase may be intermittent. Thus far it has not been possible to modify at will the zymase production of a certain race of yeast, but by careful cultivation at low temperature something may be accomplished. The same experience is sometimes made with pathogenic microörganisms. While it is usually possible to restore the virulence of such a microörganism by passing it through an appropriate animal, sometimes the culture has become so altered by prolonged cultivation upon artificial media that it is difficult to restore it to virulence. What we do not know is: what are the particular factors that determine the degree of production of zymase and endotryptase respectively? In my experience, which has been solely upon local material and entirely unsatisfactory, the proteolytic ferment has been very disproportionately active. And even with rather high concentration of the ferment the results have always been so irregular as to resemble those experiments first done by the Buchners and repeated by many others, in which with high dilutions of an otherwise active ferment the results become entirely irregular and incapable of any interpretation.

Herzog has since reported the results of a second study of alcoholic fermentation, using zymase. His results were very irregular; he did not succeed in securing a zymase sufficiently rich in the alcoholic ferment and poor in glycogen and endotryptase to warrant the interpretation of the results from the kinetic point of view. Different preparations of zymase, as first pointed out by the discoverers themselves, vary widely in enzymic power, and it is to be expected that with some preparations quantitative experiments are impossible.

Slator measured the progression of an alcoholic fermentation by the estimation of the pressure of carbon dioxide. He found the transformation quite independent of the substrate concentration, but closely proportional to the mass of ferment. In very weak solutions of sugar the transformation was roughly proportional to the substrate concentration. From this Slator concludes that the reaction is not one of the first order. The temperature coefficient he determined to be high, though deminished with increasing temperature. The values given are much too high for diffusion velocities.

Gromow has recently published the results of a study of fermentation with zymase, illustrating by the variations from the results of Herzog the uncontrollable difficulties that attend the study of this ferment. He determined first that notable quantities of products were evolved from the glycogen, which the extract first splits and then ferments to alcohol; and that the reaction was identical in an atmosphere of hydrogen and of air. He made the new observation that a false equilibrium could be established in the system; and that while the addition of more ferment would reinaugurate the reaction, the addition of more sugar would not. The addition of the products to the system early in the reaction exerted an accelerating reaction; that is, in this fermentation we have a tendency to autocatalysis. The transformation was a little less than proportional to the mass of substrate, at high dilutions, but otherwise the mass of substrate seemed to have little influence on the rate of transformation. The fermentation was a little less than directly proportional to the quantity of ferment. High concentrations of sugar were found to depress somewhat the proteolytic ferment. The

very interesting observation was made that quinine exerts a very depressing influence upon the endotryptase at concentrations that do not affect the activity of the alcoholic ferment; thus it was in some measure possible to reduce the autodigestion during the course of an experiment. Apart from the independence of the rate of transformation of the substrate concentration, these findings agree better with our general conceptions of catalysis than did the results of Herzog.

Aberson has published a noteworthy study of alcoholic fermentation. He found the transformation quite closely proportional to the mass of sugar, the constants tended to increase, and a better concordance was secured when the empiric equation of Henri ($C = \frac{1}{t} \, l \, \frac{A+x}{A-x}$) was employed. He found the acceleration proportional to the mass of ferment. He observed also under certain circumstances an equilibrium, such as has been noted in many fermentations, and was able to obtain data tending to suggest an actual reversion. The data tending to suggest the reversion of the alcoholic fermentation do not, however, carry conviction. The reaction is one that takes place in two phases. It will be recalled from the earlier work of Tammann and Nernst, that to accomplish the reversion of the reaction $Zn + H_2SO_4 <=> ZnSO_4 + H_2$ the hydrogen concentration in the system had to exceed eighteen atmospheres pressure. The reversion of the reaction of alcoholic fermentation would in a similar manner require a pressure of carbon dioxide, and this pressure would need to be high. It is possible that the ferment might shift the point of equilibrium, though this would hardly occur to a very marked extent.

Euler has carefully investigated the alcoholic fermentation of glucose. He found the constants when calculated according to the regular equation were regular and fairly concordant during the first half of the fermentation, but became progressively more irregular during the last half. The constants were not uniform in different series of different concentrations; with increasing concentration the velocity was diminished. With fixed relations of substrate and ferment in the system, the velocity of reaction tended to be directly proportional to the total concentration. With strong ferment, the acceleration was proportional to the

mass of the ferment. Throughout the work, however, one sees the disturbances produced by the endotryptase, and it seems probable that until some way is devised to shut out the autodigestion and also to secure preparations free of glycogen, reliable investigations upon the kinetics of alcoholic fermentation will not be obtainable. It was with high hopes that the discovery of zymase was greeted, for here, it was thought, lay the opportunity for the dynamic study of the fermentations. Up to the present, however, the experiences have been less satisfactory than with the diffusible and secreted ferments. In the yeast cell every function is centered in the one cell, and its extract contains all its active properties; while ferments isolated from individual parts of higher plants or secreted from specialized glands in animals naturally present a greater election and specificity in their contents and functions.

Relation of ferment mass to acceleration. In the studies of Gromow, Aberson, and Euler the acceleration was found to be in general proportional to the quantity of ferment in the system. It is to be expected that the activity of the endotrypsin would make measurements irregular.

Reversion of reaction. Apart from the qualitative work of Aberson, I know of no published study of the reversion of alcoholic fermentation, though this was in a sense anticipated by Lavoisier. As van't Hoff has pointed out, such a reversion would need to be accomplished under pressure of carbon dioxide. What will make the reversion difficult is the destructive action of the alcohol upon the ferment. Since a long time would be required for the reversion, the ferment would be unable to survive the influence of the alcohol.

Influence of temperature. The influence of increase in temperature cannot be judged, because of the action of the proteolytic ferment. That the transformation is increased with temperature is known, but the digestion of the ferment by the endotryptase is also greatly accelerated, so that the measurements are very irregular. The temperature optimum of alcoholic fermentation by zymase was not definitely determined by the Buchners. The relations are in fact exceptionally intricate on account of the presence of the proteolytic ferment. As low a temperature

as 16–18° seems the most favorable, though many facts indicate that were the zymase freed of the endotrypsin, the temperature optimum would be much higher. The findings are, furthermore, inconstant for different concentrations of substrate and of ferment.

LITERATURE.

Amylase.

Bourquelot. C. r. Acad. Sc., *105*-576.
Brown & Glendenning.[1] Proc. Chem. Soc., *18*, 42.
Brow and Glendenning.[2] Jour. Chem. Soc., *81*, 381.
Brown & Heron. Ann. d. Chem., *199*, 165.
Jour. Chem. Soc., *35*, 596.
Brown & Morris. Jour. Chem. Soc., *55*, 462-*69*, 709.
Cremer. Breichte, *32*, 2062.
Effront. C. r. Soc. Biol., *57*, 234.
Fernbach & Wolff. C. r. Acad. Sc., *137*-718; *138*-819, 849; *139*-1217; *140*-1403, 1547, 1067.
Foa. C. r. Soc. Biol., *58*, 867, 1000.
Henri.[1] Lois gén. d. Diastas., pp. 113.
Henri.[2] Ibidem., pp. 118.
Henri.[3] Ibidem., pp. 76.
Lea. Jour. Physiol., *11*, 234.
Lintner & Duell. Berichte, *26*, 2533.
Maquenne & Roux. C. r. Acad. Sc., *140*, 1303.
Moreau. Roy. Soc. Brux., 1904, 112.
Morris & Glendenning. Jour. Chem. Soc., *61*, 689.
Musculus. Zeitschr. physiol. Chem., *2*, 188.
Pawlow. Die Arbeited der Verdauungsdrusen, 1898.
Piloche.[1] C. r. Soc. Biol., *58*, 952.
Piloche.[2] C. r. Soc. Biol., *59*, 260, 263.
Roux. C. r. Acad. Sc., *140*, 943.
Sacharoff. Wratsch, 1904, No. 17.
Sheppard. Proc. Chem. Soc., *22*, 64.
Wolff & Fernbach. C. r. Acad. Sc., *140*, 95.
Wohl. Berichte, *23*-2103.
Wroblowsky. Zeitsch. physiol. Chem., *24*, 173.

Invertase.

Acree & Hinkins. Am. Chem. Jour., *28*, 370.
Armstrong.[1] Proc. Roy. Soc., *73*, 500, 516.
Armstrong.[2] Ibidem., *73*, 526, *74*, 195.
Armstrong.[3] Ibidem., *73*, 500, 516.
Armstrong.[4] Ibidem., *76*, 506, 592.
Bardendrecht. Zeit. physik. Chem., *49*, 456.
Bourquelot. C. r. Acad. Sc., *126*, 1045-*133*, 690-*135*, 399-*136*, 762.
Brochin. Jour. d. Phar. et Chim., *20*, 300.

Brown. Trans. Chem. Soc., *81*, 373.
Brown & Glendenning. Jour. Chem. Soc., 1902, 388.
Duclaux. Ann. Pasteur., *12*, 96. Traite de Microbilogie, II.
Dunstan. Zeitschr. f. physik. Chem., *49*, 59; *51*, 732.
Edlefsen. Muench. Med. Wochser., 1904, 1585.
Emmerling.[1] Berichte, *34*, 600, 2206, 3035.
Euler. Zeitschr. physik. Chem. *36*, 641; *40*, 498.
Fawsitt. Zeitschr. physik. Chem., *48*, 259.
Fischer. Zeitschr. physiol. Chem., *26*, 81.
Fischer & Armstrong. Berichte, *35*, 3151.
Hafner. Zeitschr. physiol. Chem., *42*, 1.
Henri.[4] Zeitschr. physiol. Chem., *39*-194; *51*, 19.
Henri.[5] Lois générales des Diastastes, 1903.
C. r. Soc. Biol., *57*, 171.
Herzog. Zeitschr. physiol. Chem., *41*, 416-*43*, 222.
Zeitschr. allgem. Physiol., *4*, 163.
Hill. Jour. Chem. Soc., *73*, 638.
Jour. Chem. Soc., *83*, 578.
Jodblauer & Tappeiner. Muench. Med. Wochsch., 1905, 1906.
Kastel & Clark. Amm. Chem. Jour., *30*, 422.
Knoblauch. Zeitschr. physik. Chem., 22, 268.
Lobry de Bruyn & van Erenstein. Berichte, *28*, 3078, 3085.
Rev. Trav. chim. Pays-Bas., *14*-116, 203; *16*-262.
Osborne. Zeitschr. physiol. Chem., *28*, 399.
O'Sullivan & Thompson. Trons. Chem. Soc., *57*, 834.
Plazak & Huesek. Zeitschr. physik. Chem., *47*, 733.
Rudorf. Zeitschr. physik. Chem., *43*, 275.
Sacharoff & Sachs. Muench. Med. Wochsch., 1905, No. 7.
Straub. Arch. exper. Phar. u. Path., *51*, 385.
Tammann.[1] Zeitschr. physik. Chem., *18*, 426.
Tammann.[2] Zeitschr. physiol. Chem., *16*.
Visser. Dissertation, Amsterdam, 1904. Zeitschr. physik. Chem., *52*, 257.
Wohl. Berichte, *23*-2103.
Wroblowsky. Bull. Acad. d. l'Acad. d. Sc. Cracovie, 1901.

Emulsine.

Armstrong. Proc. Roy. Soc., *73*, 520, *74*, 188.
Emmerling. Berichte, *34*, 3810.
Fischer. Berichte, *28*, 1509.
Henri.[6] Lois gén. d. Dias, 101.
Herissey. Rech. sur. l'Emulsine Diss., Paris, 1899.
Herzog. Konikl. Akad. v. Wepenschappen, Amsterdam, 1903.
Pottevin. Ann. Pasteur, *17*, 31.
Tammann. Zeitschr. physik. Chem., *13*, 25-*18*, 426.
Zeitschr. physiol. Chem., *16*.
Visser. Zeit. f. physik. Chem., *52*, 257.

Alcohol.

Aberson. Rev. Trav. chim. d. Pays-Bas., *22*, 78.
Baeyer. Berichte, *3*, 73.
Bardendrecht. Zeit. physik. Chem., *49*, 456.
Brown. Jour. Chem. Soc., *61*, 368.
Buchner & Hahn. Die zymasegaerung, 1903.
Buchner & Meisenheimer.[1] Berichte, *37*, 417; *38*, 620.
Buchner & Albertoni. Zeitschr. physiol. Chem., *44*, 206.
Zeitschr. physiol. Chem., *46*, 136.
Cochin. C. r. Acad. Sc., *96*, 852.
Duclaux. Ann. Past., *10*, 168.
Dumas. Ann. Chim. e. Physik. (5), 3, 57.
Erlenmeyer. Jour. Prakt. Chem., *71*, 382.
Euler. Zeitschr. physiol. Chem., *44*, 53-*45*, 435.
Gromow & Grigoriew. Zeitschr. physiol. Chem., *42*, 229.
Harden.[1] Jour. Chem. Soc., May, 1901.
Harden.[2] Jour. Inst. of Brewing, 1905, 2.
Harden & Young. Proc. Chem. Soc., *21*-189.
Herzog. Zeitschr. physiol. Chem., *37*, 149.
Lobry de Bruyn & van Erenstein. Berichte, *28*, 3078, 3085.
Rev. chem. Pays-Bas., *14*, 116, 203, *16*, 262.
Maignon. C. r. Acad. Sc., *140*, 1063, 1124.
Mazé. Ann. Pasteur, *18*, 535.
Miquael. C. r. Acad. Sc., *111*, 397.
Rodrigues Carrondo. Rev. Acad. Cien., Madrid, *1*, 217.
See also Seifest & Reisch. Cenbl. f. bakt. u. Parasit., II, *12*, 574.
Stoklasa. Berichte, *36*, 632-*38*, 664.
Beitr. z. chem. Physiol. u. Path., *3*, 360.
Oest. Chem. Zeit., *8*, 273.
Ber., *36*, 622; *38*, 664.
Beitrage zur. chem. Phys. u. Path., *3*, 460.
Centbl. Physiol., *17*, 465.
Pfleuger, *101*, 311.
Arch. Hyg., *50*, 165.
Centbl. f. Bact., II, *13*, 86.
Ber. dtsch. bot. Ges., *22*, 358, 460.
Slator. Proc. Chem. Soc., *21*, 304.
Tammann & Nenst. Zeitschr. physik. Chem., *9*, 1.
Wohl & Oesterlein. Berichte, *34*, 1139.

THE PROTEOLYTIC FERMENTS.

The proteolytic ferments are most widely distributed. They exist in all the invertebrates. First isolated from plants by Gorup-Besanez, they are now known to exist in all seeds and grains, and in many leaves and roots of different plants. The ferments in the seeds are usually active to a high degree only during the period of germination. Non-germinating seeds, however, contain peptone (Mark). The sap of certain trees such as the papaya is exceedingly rich in proteolytic ferment; indeed knowledge of this ferment may be said to be the most antiquated of any ferment, since it was described by Griffith-Hughes as early as 1750. The juice of the fig tree and its fruit and the juice of the pineapple also contain active ferments, and there can be no doubt that were fruits and vegetables systematically examined, proteolytic ferments would probably be found in all. These ferments are also to be found in many yeasts and moulds; they are present in all the yeasts of alcoholic fermentation, and probably constitute an invariable constituent of the cryptogrammes. They have also been so often found in all kinds of bacteria, both saphrophytic and pathogenic, that we are justified in assuming that all bacteria possess them. A perusal of the descriptions of these various proteolytic ferments of vegetable origin would tend to the dictum that they were all separate varieties; but a careful consideration of the methods employed in the studies will convince one that as a matter of fact the data are not sufficient in quantity and specific in quality to warrant any statements on classification, though they present general properties that ally them to trypsin. Vines considers plant trypsin to be composed of two ferments, one active with the coagulable and unhydrated higher proteins, the other, like erepsin, active in the cleavage to amido acids.

Proteolytic ferments have been found, with few exceptions, in all the marine and terrestrial lower forms of life. Krukenberg

failed to find them in some coelenterates, Cohneim in some echinoderms, and Fermi and Repetto in parasitic vermes. These negative results are difficult to explain, since we do not understand how the protein assimilation is possible without a ferment. In all probability, there are in these animals peculiarities in the secretion, so that the ferments escaped detection with the methods employed.

In the higher mammalians proteolytic ferments have been determined in the gastric secretion (long recognized as a peculiar phenomenon, but first described by Spallanzani and Schwann) in the pancreatic secretion (described by Corvisart, and named by Kuehne), in the succus entericus (discovered by Cohnheim), and in the tissues and fluids of the body. Both pepsin and trypsin are described in the urine. The tissue ferment is supposed to be of the nature of trypsin, and the erepsin also resembles this ferment, though, according to its discoverer, it is not able to digest coagulable protein, but is especially active in the cleavage of peptone to amido acids. The tissue enzymes are of the type of trypsin, and are currently termed the uro-tryptic, haemo-tryptic, hepato-tryptic, spleno-tryptic, etc., ferments. The proteolytic ferment described in milk by Babcock and Russell has been definitely confirmed by Vandervelle, de Wolle, and Sugg; it is also of the type of trypsin. Vernon has recently adduced evidence tending to show that erepsin is present in all tissues. The tissue ferments that are active in the aseptic autolysis of organs and tissues yields the same products that are to be obtained when the fermentation is accomplished by pepsin and trypsin. The study of the products of digestion during recent years have taught us that the distinctions between pepsin and trypsin lie in the differences in the conditions favorable to their action and in the rapidity of their acceleration, not in the chemical nature of their products, which have been shown under proper conditions of experimentation to be quite identical. Apart from the studies of Cohnheim upon the conversion of peptone into amido acid by erepsin, the studies upon this ferment as well as upon the tissue ferment have been so fragmentary as to be of little value in a consideration of ferment action from the point of view of these lectures, so that we will confine ourselves to pepsin and trypsin.

These ferments are not secreted as finished enzymes, but in the state of the zymogen. The peptzymogen is converted into pepsin by the action of the hydrochloric acid; the trypzymogen is converted into trypsin by enterokinase. They are, however, convertible experimentally by extracts of disintegrated cells of any kind; thus extracts of the powdered gastric mucosa and pancreas are active, though they have had no contact with hydrochloric acid and enterokinase. Larguier des Bancels has shown that both electrolytes and colloids can activate trypsinogen. The pure secretion of the pancreatic duct is entirely inert, as stated by Haidenhain, and confirmed for the dog by Pawlow and for man by Glaesser. This conversion of the trypzymogen into trypsin really constitutes a fermentation, as stated by Pawlow and quantitatively confirmed by Bayliss and Starling. The reaction may be stated thus: trypzymogen + water = trypsin. The reaction is one of hydrolysis; it occurs slowly in pure water, and is accelerated by many substances, especially by enterokinase. The current attempts to define the enterokinase as a co-ferment in the sense of Bertrand, as a sensibilator in the sense of Bordet, or as the amboceptor in the sense of Ehrlich, do not rest on objective grounds.

Proteolytic ferments present wide degrees of variation in different preparations. A comparison of the different commercial preparations on the market will illustrate this fact in a striking manner. Pepsin and trypsin will present wide variations when prepared from the same extract by different methods of precipitation. These variations are three-fold. Firstly, variations in enzymic strength; different preparations will vary as much as 300 per cent. Secondly, variations in the resistance to hydrolysis; some are very easily destroyed during the course of a digestion, others are quite resistant. Thirdly, variations in the temperature optimum. Different preparations may vary as much as 30° to 45°. This factor affects of course both the enzymic strength of the preparation and its resistance to hydrolysis. When to these variations that occur in the ferment prepared from one extract we add the varying strength of the original extractions or secretions, there is small wonder that the experimental variations are so striking. They indicate also how impossible it

is to measure the enzymic strength by any one method, above all by the Mett method of linear digestion in capillary tubes.

The reaction of protein fermentation. The fermentation of the albuminous substances is an act of hydrolysis. As has long been known, all proteins when heated in pure water are more or less rapidly hydrolyzed. This alone indicates that at all temperatures the hydrolysis is in slow progress. The older authors had noticed that when coagulable protein was long preserved it was prone to lose its property of coagulability. This slow auto-hydrolysis, which we may be certain always occurs when an albuminous body is suspended in water, may be experimentally demonstrated, but since the velocity is very low, a long time is required. I have preserved pure sterile globulin in distilled water for eighteen months, and at the end of that time determined that not only had the degree of coagulability diminished, but the globulin could not all be precipitated by saturation with magnesium sulphate or half saturation with ammonium sulphate, a portion was precipitable only by saturation with ammonium sulphate at high temperature. Globulin had obviously been hydrolyzed to the state of proteose. I have also determined that leucine may be recovered from a sterile solution of casein (really a suspension) in pure water, and that arginine may be recovered from a solution of protamine sulphate in pure water—both after the lapse of a year or more. An organ may be sterilized by being boiled, which will also destroy the tissue ferments, and after the lapse of months amido acids may be recovered from the tissue. Pure neucleo-proteid may be preserved stirile in pure water, and after a long time purin bodies may be isolated from the solution. These auto-hydrolyses of protein constitute the reaction of which fermentations as well as the acid cleavages constitute the accelerations. The agent of this auto-hydrolysis we assume to be the dissociated hydrogen of water.

The qualitative reactions involved in the hydrolysis of an albuminous body have been studied in part through the aid of digestions, in part by means of the cleavage with acids. The relations in the case of acids are simpler and more easy of control than in the case of ferments, and the most recent evidence is that the results are in both procedures quite the same. In considering

the hydrolysis of protein, it must be recalled that we are limiting our consideration to what we term common protein, excluding neucleo-proteids, glyco-proteids, and such bodies that contain definite complex structures not in themselves protein.

In general terms the hydrolysis of a protein may be said to pass through the following stages: Original albumin, non-coagulable albumin, primary proteose (possibly several), secondary or deutero-proteose (probably several), peptone, an unnamed sub-pepton (possibly identical in the fermentations with the polypeptide of Fischer), poly-amido-acids, grouping under this term the hexon bases, di- and mono-amido-acids. The amido acids are not hydrolyzed to lower simpler bodies, but they may by energetic oxidation and reduction be converted into carbon dioxide and ammonia, while bacterial oxidations sometimes convert them into basic amines. The sulphur of the molecule is contained in the substance cystin, which is the disulphide of *a*-amino-thio-oxyproprionic acid. This general scheme demands a closer consideration of the several steps.

The first stage, the conversion of the albumin into non-coagulable protein, is not observed for all proteins, but is encountered with many and is typically represented by the liquefaction of fibrin. In all probability the process cannot always be compared to the conversion of raw into soluble starch, or to the abolition of gel properties. In the case of gelatine, we know that a moderately prolonged heating will so lower the gel-point as to make the preparation a sol at all ordinary temperatures; and yet there is no evidence that the protein has suffered any chemical change. The simple combination of protein with acid or alkali will abolish the property of coagulation, though this may be recovered by neutralization. Possibly the abolition of the property of coagulation may correspond simply to the disruption of some chemical combination favorable to this property, or to the establishment of some chemical combination unfavorable to it. In other instances, however, the alteration is due apparently to some change in the colloidal properties of the protein. As a physical fact, we know of so many instances of the conversion of hydro-gels into hydro-sols without demonstrable chemical transformation, that we are not warranted in assuming that hydrolysis plays any

rôle in the transformation of coagulable into non-coagulable protein. I do not mean by this to state that coagulation is identical with gelification, but physically the analogy between the two is sufficiently strong to enforce the reservation regarding the relation between hydrolysis and decoagulation.

The classification of the proteoses rests almost entirely upon the results of fractional precipitation with different salts. This is one of those subjects concerning which the more one reads the less one knows. At the conclusion of the studies of Kuehne and his students, the subject seemed clear; a little later Hoffmeister and his earlier students threw some confusion into the classification. Pick, however, disorganized the Kuehne scheme entirely, and more confusion has since been introduced by Zunz and Kutscher. When one attempts to repeat these studies, one realizes why this confusion exists. It is the repetition of the experiences with the dextrines. The numerous investigators have been attempting to define chemical entities under conditions that render the demonstration of such entities improbable and the actual occurrence indeed doubtful. For this there are two reasons. In the first place, it is not probable, even granting that there may be, let us say, five different proteoses, that these are formed seriatim and that their chemical and physical qualities are so distinctive that they may be readily separated and defined. And secondly, since colloids vary so widely in their behavior depending upon their chemical experiences, it would be but natural to suppose that the albumoses would exhibit similar variations, depending upon the colloids from which they sprang and the manipulations to which they had been subjected. Precipitation of these substances by salts is less a chemical than a physical precipitation, in which the coefficient of distribution plays a predominating rôle. The fractional precipitation of albumoses could not therefore be expected to yield exact results, since under variable conditions of concentrations the process is represented by a gradient line. As a class proteoses are non-coagulable, though some of them incline to agglutination on cooling of their hot solutions; they yield all the color reactions of albumin, are precipitable by the heavy metals, but are much less sensitive to saline saturations, though they are all precipitated by zinc and ammonium

sulphates under appropriate conditions. They do not diffuse, and are strictly amorphous bodies. Their molecular weight is much lower than that of the original albumin.

The peptones are bodies of still lower molecular weight; they have some power of diffusion through membranes; they are still precipitable with the heavy metals, especially with compounds of mercury, iron, tungsten, and wolfram. They respond to the Millon and Biuret tests, but are less inclined to the other color tests for protein. While all the higher proteins are more or less inclined to denaturation on contact with alcohol, peptone is not so affected. Pure solution of peptone will conduct the current appreciably, the first body in the series to do so. The chemical combinations of peptone are more salt-like and less colloid-like than the combinations of the proteoses. The distinction of two classes of peptones, anti- and hemi-peptones, has not been maintained.

The sub-peptone stage has not been well studied. It has been long observed, but was first chemically defined by Fischer.[1] In his studies upon the products of the acid hydrolysis of protein, Fischer regularly recovered certain amido-acids, phenylalanine *a*-pyrollidin carboxylic acid, which were not to be found among the products of an ordinary tryptic digestion. He then turned to the amorphous residue that remains after a tryptic digestion following the removal of the amido acids, and submitted the gum-like substance to an acid hydrolysis (after purification by precipitation with phosphoralfronic acid), with the result that the missing amido-acids were recovered. Whether this amorphous substance, which is surely not a chemical individual, represents a residue of the protein molecule that the ferment cannot readily split, or whether it represents a combination of the products of the digestion, we do not know. It is possibly identical with the antipeptone of Siegfried.

The poly-amido acids (arginine, lysine, and histidine) are formed in an acid hydrolysis of the proteins when the reaction is not too energetic, otherwise di- and mono-amido acids seem alone to be formed. In the case of certain simple proteins, the protamines, the regular cleavage by acids leads to these hexon bases. The protamines are in some respects lower proteins than

peptone; they diffuse more rapidly, form well defined salts subject to electrolytic dissociation, respond to no color test but the biuret (which may apply to an amido acid), and form with organic anions stable physiological compounds; at the same time their molecular weight is greater than that of the lowest peptone. The poly-amido acids are not hydrolyzible by proteolytic ferments, but appear to be amenable to other ferments, and Kossel[1] has described in the liver a ferment that will greatly accelerate the cleavage of arginine into urea and ornithin.

The mono-amido acids constitute the greater portion of the products of a protein hydrolysis. Ferments act in this regard in general the same as acids, only less energetically. The statement is current that pepsin cannot accelerate the hydrolysis of peptone to amido-acids, but the contrary has recently been shown (Langstein). Trypsin always effects the production of large quantities of amido acids, and the more these are studied the more nearly the list approximates that determined for the acid hydrolysis. The recent studies of Emil Fischer[2] have already advanced our knowledge of the final products of protein hydrolysis. Through the discovery and elaboration of an ingenious method for the isolation and separation of amido acids, he had been enabled to place these investigations upon a quantitative plane; and since he has worked with large quantities of material, his results have for the first time exposed a perspective in this subject. Fischer has shown that among the products of the acid hydrolysis of protein the following are regularly encountered: leucin, tyrosin, aspartic acid, glycocoll, alanin, an amidovalerianic acid, glutamic acid, phenyl-alanine, cystine, serine and oxy-*a*-pyrollidin-carboxylic, pyrollidin-carboxylic acid, and tryptophane. In these substances Fischer and his students have been able to recover more than three-fourths of the nitrogen of the protein molecule, and when one takes into consideration that the methods do not pretend to be quantitative, one is driven to the conclusion that the group represents in all probability in an approximate manner the quantitative yield of the products of protein hydrolysis. These observations were made upon several different proteins—casein, egg albumin, globulin, edestin, hemoglobin, and fibrin. Now these same bodies have all been found

in tryptic digestions, and nearly all of them in peptic digestions. In both of these, however, the hoxon bases appear; in the acid hydrolysis they are less prominent. In the peptic and tryptic digestions may be found in addition cystin, oxyphenylaethylamine and pentamethylendiamine. The latter two are probably derived from ornithin by bacterial fermentation, and do not thus represent primary products of digestion. It is more difficult to secure a complete hydrolysis with trypsin than with acids; it is still more difficult to secure complete cleavage with pepsin, but they can be secured, and the differences seem only those of degrees.

These amido acids have lost all the qualities of proteins. They are crystalline, not colloidal, are usually basic, form well defined salts and especially good esters, and conduct themselves in every way as do the synthetic bodies of the same composition. Their constitution and molecular configuration are in many instances not understood. They contain a much higher percentage of nitrogen than do the proteins, and this regular accumulation of nitrogen in the molecule as the scale is descended as a fact of meaning.

The elective affinities of these ferments as between different proteins in the same system have not been well investigated. Gompel and Henri have shown that if raw and coagulated egg albumin are placed with trypsin in the same system, the former protein will be first digested. The current notion that raw egg albumin contains an anti-trypsin is an error. Pepsin is not able to digest protamines, mucin, chitin, or keratine; trypsin is not able to digest reticulin. Pepsin is not able to digest the synthetic polypeptides of Fischer; trypsin digests many. According to Gonnermann, trypsin is able to split acetamide, acetanilide, and formanilide. Attempts have been made to show that ferments are elective towards their own species, in the sense, for example, that a trypsin would digest the casein from its own species more easily than from a different species. These hypotheses have not been confirmed.

The modus operandi of the hydrolysis of protein is entirely unknown. Information on this point is for the immediate future to be hoped for largely through the Fischer studies on the hydrolysis of the synthetic peptides. Many of these substances are

digestible by trypsin, none so far known by pepsin. Thus far the studies of Fischer and Abderhalden have indicated only a few general suggestions bearing upon the relations of the reaction. They have found that there are structural relations of sometimes decisive importance. Thus alanyl-glycin (CH_3.CH(NH_2).CO.NH.CH_2.COOH) is digestible, while the isomer glycyl-alanine (NH_2.CH_2.CO.NH.CH(CH_3).COOH) is indigestible. Those dipeptides are best digested in which the alanine figures as the acyl. When oxy-acids, such as tyrosin, isoserin, and cystin, are attached to the end of the chain, digestion is favored. Racemic bodies they found to be digested asymetrically. As a general rule, the longer the chain of amido acids, the more readily was the compound digested. It must be confessed that these facts throw little light on the fermentation of protein, and none on the reaction of hydrolysis itself.

Relation of substrate mass to reaction velocity. It is my conviction that all the work on the quantitative relations in the fermentation of protein is of doubtful value. Through the maze of more or less conflicting data, one can detect the main fact that in the center of the numerous variables and factors stands the law of mass action and the phenomena present in an approximate way a conformity to the theory. My doubt of the validity of the published researches is based upon the conviction that in all the studies a proper measurement has been lacking. The equation of the reaction demands as the first postulate that the measurement of the work done in a unit of time shall mark a unit of work; the differential of transformation and the differential of time constitute the basis of calculation. In no work yet published, including of course my own, has the method of measurement been adequate to mark and determine the actual transformation. I have reviewed all these methods, and the errors run from 10 to 50 per cent. It will be of advantage to review them in detail.

Many investigators have measured the decoagulation of the substrate. Thus Vernon[2] measures the undissolved fibrin; others have measured the undissolved coagulated egg albumin, the ungelible gelatine, etc. Whether one weigh the unaltered protein, determine it by centrifugation, or by a determination of the

nitrogen, the analytical error is large. But the chief error lies in the assumption that the ferment first converts the hydrogel into a hydrosol as the first stage of work, and that the next stage is not begun until this one is completed. This error deprives this method of measurement of theoretical validity. With carefully controlled conditions of work, each of these procedures may seem to yield fair results, but when one compares series in which the different determinations have been employed, the fluctuations will be seen to be large and irregular. That this method may be used for physiological work is not to be denied. Vernon in particular has obtained with it many valuable facts of physiological bearing, but it is not adapted to physico-chemical research.

Similar to the above is the method of estimating the amount of substrate that has retained its coagulability by heat or precipitation by salt (Weber and Thomas). This aims to measure the conversion of globulin and albumin into their albumoses, under the assumption that this is completely accomplished before any transformation of the proteose is begun. All that has been said of the previous method applies here.

Another method consists in the estimation of the precipitable protein, using ammonium or zinc sulphate, on the assumption that the albumoses may in this manner be separated from the true peptones and amido acids. This is founded on the erroneous assumption that the albumoses may be thus separated from the peptones; the methods of saline precipitation are not reactions of combination, but are fractional procedures resting largely on the coefficient of distribution. The method yields contradictions even in qualitative work. In addition it is theoretically an invalid method, because the albumoses are not converted into peptone en bloc.

In a similar manner the formation of amido acids has been utilized as the measurement. In addition to the lack of theoretical validity as a method of measurement, this method has furthermore the disadvantage that no one knows what the alleged precipitations for amido acids, such as phosphowolphramic acid and tannic acid, will do in such a mixture. Nor does one know how much amido nitrogen is contained in each protein. Even the most careful work of the Fischer school has obtained no results

as yet that might be used as a basis of calculation. With the Fischer esterification method better analytical results could be certainly secured, but the basis of calculation would be still wanting.

The measurement of the end products of a protein digestion is at present entirely impossible for two reasons. In the first place, there is no known method feasible for a fermentation experiment. And secondly the reaction cannot be completed for so long a time that the conditions of ferment maintenance are impossible.

Another method has been the method of estimating the combined acid, as has been developed by Vollhard. It has not been demonstrated that the reactions of acid to substrate is a stochiometric one, and that the lower products do not display variable relations. In fact, it is quite certain that this is the case. I have not been able to secure concordant results with the method. It lacks obviously theoretical validity, in that it also assumes a block transformation.

Schuetz first introduced polariscopy. Here for the first time the method was accurate so far as the actual reading was concerned. It depends on the assumption that the different proteins down to the stage of peptone have fixed powers of rotation, and that these are not alterable or progressive. It must be borne in mind that solutions of sugar when freshly prepared exhibit often marked fluctuations in rotation, until finally the stable condition is established. A similar condition in the course of the protein digestion would yield entirely irregular results. The method rests upon the same error common to all the previously mentioned ones, in that it assumes that the transformation from one stage to the next occurs in block. How totally this would nullify the results if, as we know, this were not true, is easy of illustration. Let it be granted that the original protein, the primary albumose, the secondary albumose and the peptone (to make no mention of other sub-products) had a fixed factor of rotation that was attained as soon as the substance was formed. How could one calculate or measure the transformation in the system when all four were present?

Closely allied to the measurement by the spectroscope is that

by the spectrophotometric measurement of the biuret reaction. The theoretical validity and the practical errors are identical in the two procedures.

The estimation of the viscosity as a measurement of the digestion has been employed by Spriggs. He states that the diminution in the viscosity ran parallel to the conversion of coagulable into uncoagulable protein. Under these circumstances it has the same value as the estimation of the coagulable protein, but is simpler of performance.

On entirely empiric grounds, Sjoquist and Henri have employed the method of conductivity to measure the fermentation of protein. Henri and his students and Bayless have obtained very good results with this method, and when on the appearance of the paper of Henri in which the method was described I repeated the experiments with gelatine, I obtained constants that were within each series quite concordant. The method is obviously based on the proposition that the digestion comprehends the transformation of a colloid into an electrolyte; a non-dissociated into a dissociated body. To apply the method properly, the conductivity of the fully hydrolyzed system should be determined, and then the equation inverted, and consider under A the full conductivity of the products and under x the increment in conductivity in the interval of time t. Of course one could compare the readings with each other, as is commonly done with the equation $C=\frac{1}{t_2-t_1}\log\left(\frac{A_1}{A_2}\right)$, nevertheless it were better, were it feasible, to invert the equation. Despite the apparently good results, the method cannot give satisfaction until the relations are worked out. It may be assumed that with each stage of hydrolysis of the protein molecule the conductivity is augmented. Now for the stages protein –> albumoses –> peptone, the increment in the known conductivity of the substances is slight. At the point peptone –> poly-amido-acid, however, there is a heavy jump in the dissociation. Now in the experiment these are superimposed; the biuret-bearing substances begin to disappear at a time when primary albumoses are still in the system. Under these circumstances the theoretical validity of the procedure is very doubtful. To add to this doubt is the fact that the conductivity in such a

complex system is not the function of the mass of dissociated electrolytes solely.

Bayliss states that in the digestion of gelatine and casinogen the chief cause of the increase in conductivity is the splitting off of inorganic constituents of the substrate molecules, for the casinogen in addition the conversion of organic phosphorus into inorganic phosphates. According to this, the digestion of an ash-free substrate would yield little increase in conductivity. This is surely not true for protamine; there is a notable increase in the conductivity, but it is not progressive, rather step-like. If the statement of Bayliss be true, the method of measurement of the electrical conductivity cannot be a valid method of measuring a fermentation unless it be shown that the relation of the inorganic constituents to the substrate molecules is a stoechimetric one, and constant in different preparations of the substrate. The theoretically to be expected influence of alteration in viscosity on the conductivity, Bayliss found to be trifling.

A theoretically valid and in all probability very delicate method of measuring the progress of a fermentation lies in the use of the differential tensimeter. I have made a number of tests, using in part the simple apparatus of Friedenthal, in part the complicated apparatus of Smits. Thus far I have obtained no regular results, but I am convinced that the causes of this are methodical and analytical, and not due to the unadaptation of the method. It is impossible to attempt the estimation of the molecular concentration by means of the freezing or boiling point.

To avoid the entire disturbance of the reaction in many stages one should employ a pure lower protein, or use as the substrate one of the synthetic peptides of Fischer. My own studies on tryptic digestion have been carried out with protamine, and with the glycyl-glycyl-tryosin. The results with the latter have been very unsatisfactory for analytic reasons. Some of the newer polypeptides of Fischer apparently offer the ideal substrate, since they are asmyetric substances, of which the cleavage is a simple reaction, and with them the progression of the transformation would be as simple and as direct as in the inversion of sugar. I have begun the study of these substances, but have up to the present obtained no results.

For pepsin I am acquainted with but one experimental study carried out on correct lines, that of Sjoqvist. He employed an emulsion of finely ground coagulated egg albumin as substrate, was very careful with the temperature and the degree of acidity, avoided too high concentrations, and maintained a regular mechanical agitation. The reaction was measured by centrifugation of the particles and the determination of the nitrogen in the clear fluid by means of the Kjeldahl method, with corrections for the pepsin; he therefore estimated the rate of solution of the suspended coagulated egg albumin. He used also the measurement by conductivity. In the early portion of the experiments the results were irregular, but soon the reaction became progressive, and the transformation then demonstrated itself as proportional to the substrate within narrow limits. The great mass of other work upon pepsin and tryptic digestion has been done with the method of Mette, using capillary tubes of coagulated albumin, and under these circumstances the relations of velocity to substrate concentration cannot be determined, since the surface presented to the ferment remains constant during the course of the experiment, and the only alteration in transformation would be due either to the inactivation of the ferment or to the stagnation of products in the lumen. Huppert and Schuetz studied the peptic digestion of ovoalbumin, and found the acceleration under constant conditions with high dilutions fairly proportional to the mass of the substrate.

Weis (see Euler) has studied the peptic digestion of the protein of the wheat. The constants were not closely concordant in one series, while in different series of varying substrate concentrations the constants diminished as the substrate concentrations increased.

Victor Henri and des Bancels have studied the digestion of gelatine and casein by trypsin. They measured the reaction by the increase in the conductivity. They found the velocity of reaction well represented by the curve for the monomolecular reaction. The constants were in fair agreement during short tests, and were furthermore in good agreement in series with different substrate concentrations. The products were found to depress the reaction to a slight extent, but the ferment was not inacti-

vated. The chief objection to this work lies in the brief time allotted to the experiments, usually not over one hour. Now there is no such thing as the digestion of the casein or gelatine within one hour; the best that can be hoped for within that time is the conversion of the gellible into the ungellible form of gelatine. The results of Henri cannot be obtained with ash-free gelatine. My own results on this point are confirmed in the statement of Bayliss previously quoted, that the increase in the conductivity is due to the splitting off of the inorganic constituents of the common gelatine. Under these circumstances, it is clear that the results of these investigators may be related not to the hydrolysis of the protein at all, but to the cleavage of some salt-gelatine complex. This would be interesting indeed, but it would not constitute a contribution to the fermentation of gelatine.

Bayliss has recently published an elaborate study of tryptic fermentation. He employed as substrate gelatine and casinogen. As method of measurement he made use of the conductivity method. He used fresh pancreatic juice, and also commercial preparations of trypsin. He found the constants calculated according to the monomolecular equation to fall rather rapidly as the digestion proceeded. When, however, the experiments with an excessive substrate concentration are eliminated, the lack of concordance in the constants is much less striking, though apparent. This falling off in the constants was not due to the destruction of the ferment. Bayliss worked with rather strong degrees of alkalinity (as high as 2 c.c. $\frac{n}{2}$ added to 6 c.c. of system), and rapid degrees of ferment destruction as well as irregular results might be expected from this condition. Tests with fibrin gave unsatisfactory and uninterpretable results. Bayliss studied in detail the destruction of trypsin both in the digestion system and in simple suspension. He was not able to obtain regular results in the auto-destruction of trypsin, was convinced that, under the conditions of his tests, it was slight during the first six hours of the experiments, and within that time did not retard the velocity. He states that powdered trypsin became weaker with time, and quoted the analogous experience of Tammann with emulsine. (Neither Tammann or Bayliss, however, controlled the water in

their preparations. Anhydrous preparations of ferments do not disintegrate under proper conservation. I have preparations of lipase, trypsin, and pepsin three years old that are as active as on the day they were sealed. But they must be free of water and reasonable free of salts.) Bayliss considered the retardation in the reaction velocity to have been due to the influence of the products of digestion. The causes of this inhibitory influence he was not able to determine in detail, but he considered it due only in part to the combination of the products with the ferment; he was not certain that some of the effect may not have been apparent only, due to the influences of the products on the conditions of conductivity.

Hedin next studied digestion by trypsin. He employed for the substrate casein, serum albumin, and the white of egg. He measured the reaction by precipitation with tannic acid, and the subsequent estimation of the nitrogen that escaped precipitation. In a word, he attempted to measure the sub-peptone nitrogen, since tannic acid is known to precipitate peptone and all proteins high in the scale. The chemical validity of the method has, however, not been established by Hedin, or by any one else. He assumed that whatever the actual relations, they were constant in the different comparative experiments. He found a general tendency to a proportionality between the substrate mass and the transformation, but the constants were not concordant in different substrate concentrations. As the substrate was diminished, however, he found that the effect per unit of casein increased as the total amount of casein diminished, and finally became constant. Obviously, with the proper concentration this means proportionality between mass of substrate and velocity of transformation. He found that dilution of the entire system had no effect upon the total work. He observed that the ferment was to some extent inactivated during the course of the digestion. The products of the reaction he found to exert an inhibitory influence.

Hedin further made the observation that the different cleavage products of protein were not hydrolyzed with the same readiness as others. If the ferment concentration be not excessive, and under the assumption that different constituents of the pro-

tein molecule are digested at different rates, "one need only assume that a protein molecule of a certain kind as well as a certain constituent of a molecule always requires the same number of trypsin time units for their digestion, and that all the different protein molecules present as well as the constituents of the same molecule are always digested in the same order." That the different sub-products might require different degrees of work for their cleavage is well illustrated by the different peptides of Fischer. Some of them are digested by trypsin with almost explosive rapidity; others of analogous chemical composition are digested with extreme slowness.

In studying the fermentation of trypsin I have employed protamine sulphate from the salmon. This body, as pointed out by Kossel,[2] is easily and completely digestible by trypsin, though not by pepsin. It may be prepared in a state of high purity, may be sterilized without altering its characteristics, and may be accurately standardized by an estimation of its nitrogen. It has the further advantage that it forms a comparatively homogeneous solution; it diffuses through membranes, conducts a current measurably, and forms well defined salts. It shares with all proteins the difficulty of a direct and reliable quantitative estimation that would be available in a fermentation experiment. On hydrolysis it is first split into a protone, which is not precipitable by saline saturation, but is still insoluble in acidulated alcohol, in which the protamine is also entirely insoluble. The products of the second stage of hydrolysis are arginine, with traces of a mon-amido-valerianic acid, alanin and pyrrolidin carboxylic acid; the known products correspond to over 97 per cent. of the nitrogen of the protamine. These products are all soluble in acidulated alcohol, so that we possess a test that enables us to determine in a rapid and decisive manner the end of the reaction of hydrolysis, though since we cannot measure the appearance of the products we cannot record the regular march of the reaction. In the study of the effects of alkalies and other substances, as well as the relations of varying concentrations of ferment to the velocity, the digestions are carried through to the end and then the times of the completed reaction compared. For the determination of the relation of velocity to the concentration of

the substrate, the method must be employed differently, since it is not possible to remove portions of the material of a digestion experiment at stated times and determine the progress of the reaction. It is not difficult to show that the reaction is one of the first order. Therefore the determination of a constant and the relations of velocity to concentration of substrate may be determined by the preparation of a series of a dozen or more flasks of constant volume, reaction, and ferment concentration, containing of the substrate respectively 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 milligrammes. Now on the assumption that the ferment is not markedly inactivated during the short duration of such an experiment, and that the products do not depress the fermentation (which can be demonstrated for the concentration employed), it may be reasonably assumed that the time necessary to digest the solution with 10 milligrammes is the same as the time that would be necessary to digest the last 10 milligrammes in an experiment with 100 milligrammes, that the difference between the times necessary for the digestion of 90 and 100 milligrammes would correspond to the time that would be necessary to digest the first 10 milligrammes in a test of 100 milligrammes, etc.; and thus one would be able to determine the A and x of the equation $C = \frac{1}{t} \log \frac{A}{A-x}$. The results of such experiments are given in the following tables. The trypsin used was a freshly prepared filtered solution of the pancreatin, according to Spatebolz, obtained from Grudbler. This is not a particularly active ferment, but it is one of constant activity for one bottle, and is rather freer of extraneous bodies than most commercial preparations. On account of the daily variations, fresh extracts of secretion cannot be employed in this work.

Substrate 0.150. Vol. 100. Ferment 0.001. T 34°.

t	15	30	45	60	75	90	105	150
C (X 10^{-4})	68	69	69	76	66	64	62	60

Substrate 0.100. Other conditions constant.

t	15	30	45	60	75	90	105	150
C (X 10^{-4})	79	84	80	76	73	70	64	68

Substrate 0.075. Other conditions constant.

t	15	30	45	60	75	90	105	150
C (X 10^{-4})	90	97	92	88	83	88	79	73

These results indicate that while there is a general proportionality between the velocity of transformation and the substrate concentration, it is not maintained, but slowly falls. This fall cannot be corrected by the use of the later Henri equation, and is therefore due to the inactivation of the ferment and not to a greater coefficient of combination between ferment and product than of ferment and substrate. By a comparison we observe again the oft noted fact that the constants are not the same for different initial concentrations of the substrate. In the essential mathematical sense therefore the direct proportionality is only spurious, since it holds but for each particular system. The explanation might be that there may be different proportions of combination between ferment and substrate, different valencies, so to speak, and that when the two are mixed the reaction proceeds according to the particular complex adjusted in that particular system. It is to be noted that the constants rise as the substrate concentration is reduced. Acid hydrolysis of protamine follows the law for a monomolecular reaction, that of soluble globuline does not; the constants fall progressively.

These results are unsatisfactory from the point of view of the theory. Not only do the constants not agree well in the different measurements in one series, but they do not agree in different series in different concentrations. The non-conformity in series in different concentrations is due to the factor of so-called enzymic intensity. The lagging of the constants is due to the destruction of the ferment. The destruction of the trypsin is an act of hydrolysis: trypsin + water = inactive products. This destruction is more or less active is simple solution in water, though in all probability positive catalysors are present in all preparations. This destruction may be accelerated by hydrogen ions, hydroxyl ions, and by colloidal platinum. The proteins protect trypsin from this destruction, even in neutral solutions. This may in all probability be presumed to be due to a combination, while in the complex trypsin-protein, the hydrolysis of trypsin does not occur. The products of the reaction down to the stage of polyamido acids protect also, but from this point the products are positive catalysors to the hydrolysis of the ferment. The hydrolysis of the ferment is greatly accelerated by increase in tem-

perature. In view of these facts, the mystery which in the text-books surrounds the destruction of ferments should be expunged. I have tested the destruction of lipase, trypsin, and amylase, and determined for them all that they are simple hydrolyses accelerated by positive catalysors and in their progress subject to the law of mass action. Tammann demonstrated this years ago for emulsine. This being true, I have attempted to apply a correction for this destruction of trypsin, using the equation of Tammann. It will be recalled that Tammann developed the equation under the assumption that the transformation of the substrate in each unit of time would be proportional to the substrate and to the unaltered ferment. Thus $\frac{dx}{dt} = C\,(A-x)\,(F-y)$. A is the substrate concentration, x the transformation in the time t, F the ferment concentration, y the ferment destroyed in the time t. The reaction for the hydrolysis of the ferment is given in the equation $\frac{dx}{dt} = C_1\,(F-y)$, which when integrated under the assumption that $y=O$ when $t=O$ is $C_1 = \frac{1}{t}\,l\,\frac{F}{F-y}$. When these two equations are combined we have: $\frac{dx}{dt} = F.C.e.-C_1.t\,(A-x)$, e being 2.718, the basis of the system of natural logarythms. When integrated under the assumption that $x = O$ when $t = O$, we have $= l\frac{A-x}{A} = \frac{C}{C_1}.F$.

When the results of the measurements of the digestion with protamine with trypsin are calculated with this equation, the results are entirely unsatisfactory. The reason is to be found in two simultaneous tests in which the destruction of ferment is measured in a fermenting system and in simple suspension in water. It will be then found that by virtue of the protective action of the substrate, the destruction is much more rapid in the simple suspension in water, and the velocity of this destruction cannot be introduced into the equation.

Further considerations might at first suggest that the question is capable of a further mathematical development. If it be assumed that the ferment is in a properly concentrated system all combined with the substrate, it is obvious that the ferment concentration for the purpose of the hydrolysis of the ferment would in each moment be inversely proportional to the substrate concentration. As the line for $A - x$ would be descend-

ing in a logarythmal curve, the line of ferment concentration for the purpose of ferment destruction would be ascending in a logarythmal curve. This curve of F would be diminished more and more as the mass of F increased and therewith the velocity for the hydrolysis of F. Thus y would be a function not only of F, but likewise of $A - x$, since the mass concentration of ferment for the purposes of its own hydrolysis would be inversely proportional to $A - x$; and the F_1 in each moment of the reaction would be a fraction of F inversely proportional to the substrate at the same moment, *i.e.*, to $A - x$. Thus $F_1 = F - (F\frac{A-x}{A})$. Then the equation of Tammann would read: $\frac{dx}{dt} = C\ (A - x)\ (F - (F\frac{A-x}{A}) - y)$, or in shorter terms: $\frac{dx}{dt} = C\ (A - x)\ (F_1 - y)$. By making a series of tests of the hydrolysis of the ferment at the corresponding concentrations as related to the t_n in the experimental measurement of the main reaction, F_1 can be determined for those times. When these corrections are inserted into the equation of Tammann, the results may be calculated and the hypothesis tested by the uniformity of the constants for the main reaction.

Now this equation does not work in practice. When the results are calculated with it, the lagging of the constants is prevented, but for it is substituted an acceleration of the constants; the defect is over-corrected. This may be taken to indicate that the ferment is not all combined with the substrate in such a system—a conclusion that has already been reached on different grounds by Bodenstein and Henri. If now we should attempt to include the equation of Henri (described under intervase) the proposition that the ferment combined with the substrate was protected from hydrolysis, while the ferment free was subject to hydrolysis and the ferment combined with the products subject to hydrolysis, we would have an equation containing five constants. Such an equation under the circumstances would be valueless, even for purposes of interpolation.

The remedy lies in another direction. We must seek less sensitive proteolytic ferments. Those of the higher plants are little better than those of the higher animals. In all probability in the lower plants, in the yeasts, or in the lower forms of marine

vegetable or mammalian life, the proteolytic ferments may be found that will carry on a simple digestion without loss of enzymic power. Known for these ferments already are their low temperature optimum and their digestive vigor. With the use of such a ferment and the employment of one of the synthetic asymmetrical polypeptides as the substratum, we may hope to show in an unequivocal manner that the fermentation of protein follows the law of mass action, and that our present irregular results are due to the influence of uncontrollable and adventitious variables. A careful comparison of the results of Henri, Bayliss, Hedin, and my own justifies the scepticism expressed in the statements introductory to the discussion of this subject. I believe the speculations of Henri, Bayliss, and Hedin will avail little until we secure simpler conditions of experimentation.

Relations of ferment concentration to rate of acceleration. This has been often studied for both pepsin and trypsin. The earliest work, that of Bruecke, led him to the result that the velocity of transformation was proportional to the quantity of ferment. Schuetz in his work found that the transformation in a unit of time was not directly as the quantity of ferment, but as the square root of the ferment. For example, if in two tests the transformations were as 2 and 3, the ferment in the two tests were related as 4 to 9, *i.e.*, $\frac{C_1}{C_2} = (\frac{con_1}{con_2})^{\frac{1}{2}}$. This relation seems to have been regularly found by the students of the Pawlow school, using the Mette method. Linnossier, however, who used the same method, could not obtain the same results. As a matter of fact, an extended experience with the method of Mette will convince any one that it is not adapted to accurate work, and that it is not the proper way of approaching the problem. Vernon[5] was able to obtain the rule in the digestion of fibrin only by making elaborate and certainly inaccurate corrections for the inactivation of the ferment. Sjqovist in the work on pepsin digestion found that the transformation was proportional to the quantity of ferment. The work of Henri indicates also that the transformation is proportional to the quantity of ferment.

Spriggs studied the peptic digestion of syntonine, using the viscometer as the method of measurement. His results were irregular; in some regions the curves approximated a proportion-

ality between ferment mass and acceleration, in other regions they tended to conform to the rule of Schuetz. Bayliss and Hedin determined for trypsin that the acceleration was closely proportional to the ferment mass if the ferment concentration be not excessive. Loehlein, using the acidimetric method of Vollhard, determined the relations for pepsin to be proportional to the square root of the ferment mass, but directly proportional for trypsin. Huppert and Schuetz were not able to obtain good results with the Mette method, but using the estimation of the secondary albumose as a method of measurement they determined that there was an approximate proportionality between the acceleration and the mass of ferment. The results of Pollok were not favorable to the Schuetz rules.

In the digestion of protamine with trypsin the rate of transformation has always been found proportional to the quantity of ferment. The quantity of ferment was varied, all other conditions being held constant. The results are given in the following table, with the average error of the average:

Table II.

Digestions at HO-optimum. Protamine 0.100. Vol. 50. t, 40°.

F.	0.008	0.006	0.004	0.003	0.002	0.001	0.0005
t	37	49	77	101	145	320	615
	38	50	82	106	170	340	680
	40	55	82	110	165	335	654
	37	51	75	102	159	313	710
	35	46	72	98	147	290	660
	36	47	71	99	148	280	630
Avg.	37	50	76.5	103	156	313	657
±	0.45	1.1	1.9	1.8	2.5	9.8	13

From these figures it is apparent that at this concentration of protamine the velocity of hydrolysis is directly proportional to the quantity of ferment. Time × ferment give the following figures:

296. 300. 309. 312. 313. 328.

Considering the acknowledged errors in the experiments, the concordance is sufficient to warrant the statement of direct proportionality. The figures tend to increase with the dilution of

the system, due to the destruction of ferment in the prolonged periods of digestion.

These experiments have been often repeated on different preparations of protamine with different preparations of trypsin, and always with the same result. An interesting method of illustrating the non-observance of any such relation as that of the so-called rule of Schuetz, is to place in different flasks ratio quantities of substrate, say 100, 200, and 300 milligrammes, and after determination of the time required for the digestion of the lowest concentration with a certain quantity of trypsin, the others are digested with those quantities of the ferment as correspond to the squares of the substrates, and under these conditions all should be digested in the same time. The results always are that the higher concentrations are not digested in the same time, but much more quickly.

Protamine.	0.050	0.075	0.100
Ferment.	0.002	0.0045	0.008
t	87	61	53
	81	60	51
Protamine.	0.100	0.150	0.200
Ferment.	0.002	0.0045	0.008
t	170	141	121
	156	106	lost.

As a physico-chemical procedure the digestion of constant solutions of a homogeneous substrate, under constant conditions, with a regular measurement of a unit of work, employing concentrations of substrate known to lie below the point of inactivation of the ferment by the products, would certainly seem to be superior to the digestion of a tube of coagulated egg albumin. And it seems strange that following the publications of Kossel and Matthews, this procedure has not been employed in studies on trypsin.

I have studied the digestion of soluble globuline with pepsin, and found the velocity proportional to the mass of ferment. The following figures will serve to indicate the results.

1/10% solution substrate
1/100% ferment in *a*
1/200% ferment in *b*

The times are those necessary to convert all globulin beyond the state of precipitation by magnesium sulphate.

a — 86 *b* — 182

In this connection it will be pertinent to discuss the rule of Schuetz (according to which the accelerating action of a ferment is not proportional to the mass of the ferment, but to the square of the mass), since it has been largely in connection with the proteolytic ferments that this rule has been developed. That this rule represents a physico-chemical law of ferment action per se, as is currently assumed by physiologists, is entirely incorrect. The fermentations under consideration are all monomolecular reactions, and no accelerator of such a reaction that acts as a pure accelerator and appears in the products unchanged and not in combination with or unaltered by the products of the reaction could have its influence expressed in such a mathematical relation as is contained in the rule of Schuetz. In those instances of fermentation in which this rule appears to be followed, something operates to invalidate the direct proportionality that ought to hold, the rule of Schuetz instead of being a rule of ferment action per se is to be regarded instead as the result of some particular determinant influence that causes a regular and quantitative deviation from the normal progression of the acceleration. For many fermentations the rule does not hold at all. Thus the digestion of protamine by trypsine, the fermentation of soluble esters by lipase, the action of rennet, and other well studied reactions yield the normal result that the acceleration of the reaction is proportional to the mass of the ferment; or, in other words, the product of the time and the ferment mass is constant for a fixed concentration of substrate. Most observers of the digestion of the higher colloidal proteins have, as above stated, found the rule of Schuetz to hold within certain limits. It is, however, not uncommon to find the rule of Schuetz to hold for certain (usually higher) concentrations, while the rule of direct proportionality holds for higher dilutions. Furthermore, in the same experiment, the rule of Schuetz may hold for the first part of the digestion, to be supplanted later by a rough proportionality. My own studies suggest that even for the digestions of the higher proteins the use of high dilutions will usually give a direct proportionality between degree of acceleration and ferment mass; the tests for the end reactions are, however, at these dilutions not sharp, and the proportionality is only approximate. The fact that prota-

mine differs strikingly from the other proteins suggests that possibly the regularity of this reaction may be due to the fact that the protamine is always digested in the form of a salt, usually the sulphate. Now this is an ordinary electrolytic salt. After its digestion. the products (the chief one is arginine) is combined with the SO_4 ion to form an electrolytic salt. One might infer that the difference in the two digestions might lie in the fact that in the digestion of the protamine the products are completely combined, whereas in the digestion of the higher proteins the products remain in the system in the form of free amido acids, unbound. That this cannot be the direct explanation is seen, however, in the fact that the situation is reversed in the case of the digestion of the different fats. In the digestion of the glycerides of acetic and butyric acids, whose products are entirely soluble (and quite strong acids), the rule of direct proportionality holds; on the other hand, in the digestion of the higher fats whose products are insoluble (and very feeble acids, the glycerine is the same for the two systems), the rule of Schuetz holds. Direct experiments indicate that the presence of glycerine and acetic acid have little influence on the action of lipase from the castor bean. One is therefore led to wonder if it is possibly anything in the colloid state that accounts for the deviation in the digestion of the two kinds of glycerides, and whether it may not be the colloidal lower proteoses and not the amido acids that are accountable for the deviation in the digestion of proteins. This inference could be tested by the digestion of pure peptone to amido acids, or by the digestion of the polypeptides of Fischer. There is no direct data bearing on occurrence of the rule of Schuetz, *i.e.,* on the occurrence of a deviation from the regular progression of the digestion; and the chief concern it holds for us now lies in the fact that it has been misinterpreted to express a rule for ferment action per se, and one that has been misemployed to maintain a distinction between ferment and catalytic reactions. Hofmeister has suggested that the rule expresses a relation of dissociation between the different components, but this has not been tested experimentally or mathematically.

Relation of temperature to reaction velocity. The velocity of a tryptic digestion is increased from 10° to 40° in accordance

with the theory, being more than doubled for every 10°. From 40° to 45° the increase is much greater in many preparations, and then there is a sharp turn in the curve, as the destruction of the ferment becomes predominant. Bayliss found that trypsin would act at zero. In the investigation of the influence of temperature, one must always determine the temperature optimum of the preparation, operate in a dilute solution, and have the system as nearly neutral as possible, though still retaining an alkaline reaction. Henri, Hedin, and Bayliss all found the same relation to increase of temperature, and I have always found it observed. The following experiment will illustrate the facts.

Substrates, 0.1% protamine, reaction N--/10000, trypsin 0.001.

Temperatures	20°	30°	40°
Times for complete cleavage	1380	680	310

The prolonged digestions tend to lag, a phenomenon due to the destruction of the ferment, even at the lower temperature.

The *reversion of protein hydrolysis* has never been certainly accomplished. The phenomenon of plastein formation belongs here. When peptones are digested with gastric contents a gelification may be observed, and this has been held to be due to the reformation of a globulin, as the body is precipitated by carbon dioxide and by magnesium sulphate, and coagulated by heat in the presence of a trace of acetic acid. Herzog, who has recently repeated the experiments, and confirmed the experience, with the addition of data showing the increase in viscosity accompanying the transformation, is strongly inclined to regard the process as a reversion of ferment action, the condensation of propeptone to a higher protein.

I have attempted in every way the tryptic and also the acid reversion of the digestion of protamine, absolutely with no result. I further tried to resynthesize *b*-naphtalinsulphoglycyltryosin, the digestible polypeptide recently synthesized by Emil Fischer, but to no result. The reversion of the fermentation means simply the condensation of amido acids with the extrusion of water. Within the last few years this question has been taken up by Emil Fischer, who has turned to this problem the wonderful synthetic powers that first accomplished the synthesis of the sugars, and then of the purin bodies. By the application

of most ingenious methods, Fischer has succeeded in condensing amido-acid along the lines of the lower proteins. Some of these bodies he terms polypeptides, and they resemble very strongly the sub-peptone stage in a tryptic digestion. Many of these bodies respond to the biuret test, and are digestible with trypsin. Just lately it has been announced that another step had been accomplished, and that bodies have in this way been synthesized that can scarcely be distinguished from true peptones. Apparently we are on the verge of the synthesis of the higher proteins, that have for years been the last resort of the vitalists. It is only proper to state that Fischer was led to these studies by the results of his investigations on the hydrolysis of proteins by acids, and the history of these studies furnished only another illustration of some advantages of a purely chemical investigation of biological problems. It is the old story—while the biologist sits contented, wrapped in the mantel of vitalism, the pure scientist harvests his fields.

While it is true that a reversion of the hydrolyses of protein under the influence of ferments has not been demonstrable, there are indications of an equilibrium in the system. No digestion of a protein is completed in the quantitative sense in any experiment in vitro. After the reaction has come to a standstill, it may, under favorable conditions, be possible to show that the ferment is still active. The further addition of ferment has never in my experience been able to reinaugurate the reaction, and this is but a confirmation of the findings of Bayliss. By a removal of the products, by the addition of more substrate, by raising the temperature or by diluting the system, the reaction of cleavage may be reinaugurated. Similar results are to be noted in the acid hydrolysis of protein. If the acid be not too strong, the reaction will not be complete. This cessation of the reaction is not due to the binding of the acid by the products. If more substrate be added, the reaction will be reinaugurated. The failure at reversion by ferment action is paralleled by failure at reversion by the aid of acids. In the case of the ferment, the destruction of the ferment in the prolonged time that might be anticipated as necessary for a demonstrable reversion might be as-

signed as the cause of the failure; for the failure with acids, no such explanation is at hand.

Conditions of action. Pepsin acts best in an acid reaction. This favorable influence of acids is due only in part to the hydrogen ion. It is true that pepsin will act measurably in any acid of appreciable dissociation, but its action is particularly stimulated by certain acids, and without any relation to their dissociation constants. Hydrochloric acid is most favorable. Next come lactic acid and oxalic acid. Sulphuric acid is very inferior to hydrochloric, phosphoric acid less so. In fact the activity of pepsin is so connected with the presence of acid that many authors consider that the real ferment is a complex pepsin-acid. Of this there is no direct evidence. Other investigators believe that the acid acts upon the protein, and that it is the complex protein-acid that is the substrate of the digestion. Theoretically, the acid might be regarded as a powerful zymo-exciter, but the peculiar dependence of pepsin upon the acid (no other ferment is so dependent upon the reaction as pepsin) makes this view seem a distant conjecture. That acid alone will split the protein is of course true, but the digestion of protein by pepsin in an acid medium is not to be regarded as the additive results of two catalysors. The peculiarly favorable influence of hydrochloric acid might, from the point of view of the acids alone, be explained either as the result of a positive influence of the chloranion, or as the result of negative influences of the anions when other acids are employed. This would rest the positive catalysis upon the hydrogen ions, and make the deviations dependent upon the positive or negative influences of the anions of the different acid. The current statements upon the most favorable degree of acidity, which are stated to be from one-fourth to three-fourths per cent., are of little value. In the first place they assume that the relation is directly as the degree of acidity, which is not true. Secondly, it is assumed that the influence is related to the ferment alone, which is not true. To determine the facts two considerations must be maintained. The substrate concentration must be held constant, because the protein combines with acids to form compounds subject to a high degree of hydrolytic dissociation. The amount of acid necessary to combine with the sub-

strate must be determined, and then the action of a constant quantity of the ferment may be determined in a series of tests containing increasing quantities of the acid. Hydrochloric acid alone has been well studied, and even here the data are not entirely harmonious. Employing egg albumin and estimating the digestion by the disappearance of the power of coagulability, my own experience with human gastric secretion has yielded the result that the activity of the fermentation increases up to about twentieth normal, and then continues with no variation up to considerably more than tenth normal, after which a rapid fall occurs. The gastric juice of different individuals, however, often exhibited rather sharp variations. Using commercial pepsin and globulin as the substrate, I have found that rather higher quantities of acid may be tolerated, but this may have been due to the presence in the pepsin of protein that would combine with acid. Marked variations were here also observed with different preparations. We thus encounter again the experience of the variability of enzymic qualities depending upon the life history of the preparation.

For trypsin the equally unequivocal statement is also made that it acts best in an alkaline medium. In a restricted sense the statement is true. But the conditions are so complex that it is doubtful whether the relation is directly referable to the reaction. Foa found the alkalinity of pancreatic juice to be N/10000, of succus entericus N/70000 KOH. That trypsin will act in dilute acid solution is an old experience; that it is very sensitive to a slight excess of alkal is also an old observation. Historically, the idea that trypsin acts best in an alkaline medium was based in part upon the idea that the contents of the small intestines were alkaline. This has been much exaggerated. As a matter of fact, the general reaction of the ilium is quite neutral. Now when one considers that the products of tryptic digestion are largely alkaline, one is led to doubt the degree of preformed alkalinity. In a study of the tryptic digestion of protamine sulphate I determined that an initial alkalinity of about one-fifteenth hundred normal constituted the optimum reaction. Since that time, pursuing the matter further with the aid of estimations of the reaction by means of the gas cell, I have learned that that concen-

tration of initial alkali is most favorable which is sufficient to neutralize about a N/1000 acid solution after neutralization of the products of the hydrolysis, which are slightly acid. This may not hold for other proteins. In any event it is clear that the optimum alkalinity is a very slight one, and that here it is simply the hydroxyl ions that act, since the hydrolides of all the alkaline act in proportion to their dissociation, except that barium depresses strongly. Vernon[1] believes the protective action of protein for trypsin to lie largely in its power of binding alkali. This is only in part true, since destruction occurs in distilled water. The alkali simply accelerates the hydrolysis.

The influence of nearly every imaginable foreign body, salts, organic substances, alkaloids, have been tested, these researches having been stimulated in the hope of determining the influence of drugs upon the digestion. Out of the mass of conflicting data, whose importance has been highly overestimated, it will suffice to state that most salts have no effect in dilute solution, but are depressant in high concentrations; that the salts of the heavy metals tend to depress; that alkaloids, alcohols, and aldehydes tend to depress; and that the purin bodies tend to accelerate. We meet here with the distinction previously observed, that some substances simply inactivate a substance, while others destroy it. Thus the neutral salts simply inactivate pepsin, and its enzymic power is restored on their removal; alkaline salts on the contrary destroy it. The true value of this work is difficult to estimate, but the human experience that our digestions are very oblivious to moderate abuse indicates that their practical application is not farreaching.

Pepsin, under conditions of favorable environment in a digestion experiment, is very resistant to destruction. Trypsin is much more sensitive. This means that the rate of the hydrolytic cleavage of trypsin is more rapid than with pepsin. Under appropriate conditions they will digest each other. Though the proteolytic action of trypsin is much more marked and rapid than that of pepsin, it dies out in an experiment more quickly. For both these ferments, however, the prolonged experiments once reported must have rested upon a fallacious foundation. It is not at all uncommon to read in the older literature of setting

aside a digestion experiment for a year or more. I have not studied pepsin in this regard, though I am certain that in a sterile mixture it would not exist a year. With regard to trypsin, I can state that direct experiments have yielded the result that with no commercial preparation of powdered pancreas will the ferment activity survive a few weeks; usually the ferment is permanently destroyed within a few days. For both these ferments the rule holds that they remain active longer, that is, resist hydrolysis longer, when engaged in digestion or in contact with protein than when simply suspended in water. When dessicated they may be preserved almost indefinitely, with no measurable loss in activity. They also bear heating when dry to over 100°.

The temperature optimum for both these ferments runs from about 40° to 45°, and above 50° there is a very rapid destruction. Up to about 45° the increase in activity follows the general law for the increase in the velocity of a reaction with increase in temperature. For different preparations of the ferments the relations may be different, and especially will the temperature optimum be changed when the ferment is acting under unfavorable conditions. Nothing could more strikingly illustrate the independence of the temperature optimum from the temperature of the animal than the fact that the pepsin of the frog has the same optimum as that of the warm blooded animals. For fishes, however, Hoppe-Seyler found a low temperature more favorable. As a whole, the temperature optimum of ferments lies considerably higher than the body temperature of the plants or animals that produce them.

For trypsin higher concentrations of the ferment yield more rapid comparative transformations. For pepsin this is not true; dilute solutions give better comparative digestion. This is in all probability due to the relations with the acid. Pepsin is quite tolerant of the presence of the products of reaction; in ordinary concentrations a peptic digestion proceeds no more rapidly in a dyalisor (the acid being kept constant) than in a flask. Trypsin, on the contrary, is sensitive to the presence of even a slight excess of the products of digestion; a digestion experiment in a dyalisor proceeds more rapidly and to a greater degree than in a flask.

Pepsin is quite resistant to the action of bacteria. This may

in part be accounted for by the fact that the acid concentration in the case of peptic digestion is an unfavorable medium for the development of microörganisms, though any one who has attempted to check the bacterial fermentation in chronic gastric disease by the use of hydrochloric acid will soon learn how weak is the anti-bacterial action of this substance. Trypsin, on the contrary, is in experiments in vitro very sensitive to the action of bacteria; to secure results in a tryptic digestion the addition of an adequate amount of an appropriate antiseptic is absolutely essential. Now this is in direct contrast to the natural environments of the ferments. Pepsin is in the stomach exposed to comparatively few microörganisms, while trypsin in the intestine is surrounded by innumerable bacteria of all kinds, particularly bacteria of putrefaction. Nevertheless the velocity of tryptic digestion in the intestine is such as to indicate that the bacteria exert no noteworthy inhibition.

Both pepsin and trypsin have been isolated in a crude state; the best preparations have been of pepsin by Pekelharing. The general methods are the same. Watery extracts are prepared, or the secretions obtained. These are then filtered, and the ferment precipitated by alcohol, and the colloid washed with alcohol. The residues are then dissolved in water, and freed of salts and other bodies by dyalysis; in this act the pepsin suffers but little, but unless it is done at low temperature trypsin will be greatly destroyed, so that this process is often omitted in the case of trypsin. The ferment may be precipitated a second time by alcohol or by ammonium sulphate. The final preparations are never pure. The purest pepsin of Pekelharing was free of phosphorus, but contained both chlorine and iron. These ferments have a percentage constitution similar to ordinary protein. They display the reaction of protein, are coagulable by heat, polarize light, and on digestion or hydrolysis yield the products usually obtained from neucleo-proteids: amido acids, pentose, and purin bases. They are absolutely non-diffusible, and although their solutions are clear and colorless, they are always colloidal and have a high viscosity for their weight concentration. They are retained to a notable extent by infusorial filters, to some extent by paper filters. The dried preparations and those precipitated

by ammonium sulphate are very stable; those precipitated tend to display denaturation. Solutions of such pure pepsin will keep for a few days at low temperature; solutions of trypsin decompose much more rapidly.

Schrumpf has described a preparation of pepsin that gave no biuret, Millon, picric acid, uranium acetate or ammonium sulphate reactions, did not curdle milk, and was an active proteolytic enzyme.

LITERATURE.

Babcock and Russell. Cenbl. f. Bact. (2), *6*, 22, 45, 79.
L. des Bancels. C. r. Acad. Sc., *141*, 744.
Bayliss. Arch. d. Sc. biol., *11*, Supp. 261.
Bayliss and Starling. Jour. Physiol., *30*, 61-82, 129.
Cohnheim. Zeitschr. f. physiol. Chem., *33*, 9.
Euler. Zeitschr. f. physiol. Chem., *45*, 422.
Fermi and Repetto. Cenbl. f. Bact., *31*, 403
Fischer.[1] Zeitschr. f. physiol. Chem., *33*, 157.
Fischer.[2] Numerous studies in the Berichte of the German Chem. Soc. and in the Zeitschr. f. physiol. Chem. during the last five years.
Fischer and Aberhalden. Zeitschr. f. physiol. Chem., *46*, 52.
Foa. C. r. Soc. Biol., *58*, 867, 1000.
Friedenthal. Cenbl. f. Physiol., *17*, 437.
Gies. Am. Jour. Physiol., *8*, XXXIV.
Glaessner. Zeitschr. f. physiol. Chem., *40*, 465.
Gompet and Henri. C. r. Soc. Biol., *58*, 459.
Gonnermann. Appotek. Zeitung, 1902, 132.
Arch. f. d. ges. Phys., *89*, 493; *95*, 278.
Hedin. Jour. Physiol., *32*, 468.
Henri and des Bancels. C. r. Acad. Sc., *136*, 1581.
C. r. Soc. Biol., *55*, 563, 787, 788, 789.
Hueppert and Schuetz. Arch. f. d. ges. Physiol., *80*, 470.
Kossel.[1] Zeitschr. f. physiol. Chem., *41*, 32; *42*, 181.
Kossel and Matthews. Zeitschr. f. physiol. Chem., *25*, 190.
Krukenberg. Mittheil. a. d. physiol. Inst. Heidelberg, *2*, 1, 261, 338, 366, 402.
Kutscher. Zeitschr. f. physiol. Chem., *23*, 115. See also Folin, ibidem, *25*, 152.
Langstein. Beitr. z. chem. Physiol. u. Path., *1*, 507-*2*, 229.
Linioissier. Jonr. Physiol. et Path. Gen., *1*, 281.
C. r. Soc. Biol., *52*, 288.
Loehlein. Beitr. z. chem. Physiol. u. Path., *7*, 120.
Marx. Zeitschr. f. physiol. Chem., *42*, 259.
Nierenstein and Schiff. Arch. f. Verdauukrank, *8*, 559.
Pekelharing. Zeitschr. f. physiol. Chem., *35*, 8.
Pélabon. C. r. Acad. Sc., *124*, 360. These, Bordeaux, 1898.
Pick. Zeitschr. f. physiol. Chem., *24*, 267-*28*, 219.
Beitr. z. chem. Physiol. u. Path., *2*, 481.
See also Hart. Zeitschr. f. physiol. Chem., *33*, 347.
Pollok. Beitr. z. chem. Physiol. u. Path., *6*, 95.
Schrumpf. Beitr. z. chem. Physiol. u. Path., *6*, 390.
Schuetz. Zeitschr. f. physiol. Chem., *9*, 577.
Siegfried. Zeitschr. f. physiol. Chem., *35*, 164.
Sjoqvist. Scand. Arch. f. Physiol., *5*, 354.

Smits. Zeitschr. f. physik. Chem., *39*, 289.
Spriggs. Zeitschr. f. physiol. Chem., *35*, 463.
Thomas and Weber. Cenbl. f. Stoffwech. u. Verdauukrank, *2*, 14.
Vandervelde, de Waele, and Sugg. Beitr. z. chem. Physiol. u. Bath., *5*, 571.
van't Hoff. Vorlesuung. u, theoret. u. physik. Chem., *1*, 202.
Vernon.[1] Jour. Physiol., 32, 33.
Vernon.[2] Jour. Physiol., *26*, 405, *28*, 375.
Vernon.[3] Jour. Physiol., *26*, 420.
Vernon.[4] Jour. Physiol., *26*, 412, *31*, 346.
Vines. Ann. Bot., *19*, 171.
Volhard. Zeitschr. f. klin. Med., *44*, 480.
Zunz. Zeitschr. f. physiol. Chem., *27*, 219-*28*, 132.

THE FERMENTATION OF FAT. LIPASE.

The fat-splitting enzyme, lipase, has a less wide distribution in the vegetable world than the ferments of the carbohydrates. Described first by Muentz, lipase is now known to be present in most seeds, resting as well as germinating. It does not seem to exist in the growing parts of higher plants. It is found in most yeasts, notably in penicillium glaucim and aspergillus, and in some bacteria, as the proteus vulgaris. Of pathologic bacteria the vibrio of cholera, the bacillus typhosus, the colon bacillus, and the bacillus pyocyaneous have the power of fermenting fats. In none of these, however, does the faculty seem prominent. Even in putrefactions, the organic and volatile acids are in large part derived from the protein and carbohydrates rather than from the fats; and it is not certain to what extent the cleavage may not have been accelerated by alkali. In the seeds rich in oils, as in castor beans, we find the highest content of lipase.

The presence of lipase in the pancreatic juice was described by Claude Bernard, although he interpreted the phenomenon incorrectly, and it was Bruecke who first explained the reaction in the process of emulsification by the pancreatic juice. Lipase is now known to exist in the secretion of the stomach (Volhard[1]), although not active in an acid medium; in the succus entericus (Balribebb), in the liver, blood serum, and therefore in all tissues (Hanriot[1]). The statement is quite current that the lipase of the liver is different from that of the pancreas, because of a different attitude towards acid and alkali. Lipase is also known to exist in many of the lower forms of life, both marine and terrestrial.

Lipase may be extracted from tissues or seeds by water after maceration with quartz sand. It confers an opalescence upon the suspension, will not dyalize, filters poorly, and is indeed retained in large part upon the filter, passes through an infusorial filter with great difficulty, and may be precipitated by alcohol. It is quite a stable ferment, even in the watery extracts of mammalian tissues the ferment is conserved to a noteworthy degree.

When dried it may be kept indefinitely. I have preparations of lipase over two years old that are as active as the day they were prepared. It is digested by both pepsin and trypsin, but is especially sensitive to trypsin. It is for this reason very difficult to isolate it from the pancreatic secretion. When a pancreatic powder or extract is so prepared as to conserve its proteolytic ferment to the greatest extent, the lipase is usually sacrificed entirely in the autodigestion. In only one of the commercial preparations of the pancreas have I been able to find any lypolytic action, and in this preparation, named Pankreon, this activity was but slight. Powders prepared from perfectly fresh pancreas, on the other hand, will display a high degree of lipolytic activity, with a deficiency in proteolytic activity. Most of the enzyme seems here to be in the state of the zymogen, but it is readily activated by the addition of a little acid. These pancreas powders are, however, inactivated in solution within a day, in much less time in fact than the lipases prepared from the liver and blood serum, which are quite stable.

Lipase is much more readily prepared from castor beans than from any other source. The ferment is best prepared as follows: The beans should be several months old, but not several years old, as is quite the custom in the markets. The shells should be removed by hand without the use of water. The seed is then macerated and extracted with alcohol-aether for a day, and then extracted for another day with aether. Following the removal of the larger portion of the fat, the material is then ground and mortared to the finest consistency and passed through a fine sieve. It is then extracted with aether through several days. It is possible within a week to secure the powder free of fat, and this is desirable in quantitative work. From the point of view of the ferment, it were in all probability permissible to leave a certain amount of fat in the power, which could then be determined and the figure added to the substrate. Since one should employ a pure fat for the substrate, it is advisable to extract all the fat. The material then presents the form of a light impalpable powder, not very hygroscopic, and perfectly conservable. It cannot be readily weighed on account of the magnetic property that causes the particles to fly apart when touched. It forms

with water a thick and quite permanent emulsion. A watery extract of this powder will exhibit lipolytic activity, though to a much less degree than the powder. The powder on suspension in water will maintain its lipolytic activity for days if the temperature be kept low. I have made numerous attempts to isolate the ferment from the powder by precipitation of the watery extract with alcohol, but always with the result that not only was the ferment weaker, but it was also very vulnerable and almost wholly devoid of that resistanee to outside influences that is a pleasant feature of the powdered seed. In the powder are of course nearly all the constituents of the seed except the fat, and a few salts and lipoidal substances that were soluble in the alcohol and aether. In this powder, even when suspended in water, the lipase seems to be protected, if the temperature be kept below 30°. This lipase works well at 20°, and as the proteolytic ferment in the bean does not seem to attack it (or to get around to it after digesting the numerous other proteins present) at this temperature, we are enabled to work with the ferment with satisfactory results. The powder contains a great deal of protein, and this seems to protect the lipase. Once prepared, the powder may be conserved for years. The commercial residues, obtained after compression, are of less value and may be of no value, owing to the heat developed during the process of expression of the castor oil. Very old seeds will also yield poor powder; low in lipase and tending to decompose. The powder should be sterile. Following the prolonged extraction with aether and alcohol, the powder is sterile, and any contamination that may lodge during the process of sieving and drying may be killed by heating the dry powder to 100° for a short time. Sinee it is possible to sterilize the fats to be employed as the substrate, one is freed of the necessity of employing antiseptics. The ferment bears such antiseptics as chloroform well, but the presence of much chloroform disturbs the emulsification. Toluol is the best antiseptic when one is required. The castor powder is not especially strong in lipolytic activity. I have seen pancreatic extracts twenty times as strong. But it is even in its action, constant in results, little disturbed by extraneous conditions in general, and very resistant to destruction. The use of this powder

for the technical cleavage of fats is now patented in Europe, and in one of the trade descriptions of the process the statement is made that the powder may be used over and over again. That is of course putting the case entirely too strongly, but it is possible to secure a powder so stable that one can carry through tests that occupy several weeks at 20° without any loss in the enzymic power—a circumstance of great importance in the employment of a ferment for research purposes. For these reasons I have latterly confined myself to the use of this ferment, and have been able to obtain results that I at least could not obtain with animal ferments.

This vegetable lipase acts best in an acid reaction. The powder itself is acid. I have never been able to prepare a neutral powder. The powder in the tests to be described was acid in the proportion 1 G. = 0.4 c.c. N/10. The degree of the reaction should be slight, though the ferment will tolerate without inactivation other than reversional quite a degree of acidity due to such slightly dissociated acids as acetic and butyric acids; for example, a thirtieth normal. Indeed the products exert an autocatalytic influence, though it cannot be affirmed that this is due to the acid alone. Some of the acid is undoubtedly combined with the protein of the powder, which probably explains a fact that is to be mentioned later, that this ferment fails by a little the ordinary point of equilibrium for the system under experimentation. Many authors in writing upon the favorable or unfavorable influence of alkali do not seem to realize that unless the alkali be added in quite notable amounts it will be neutralized by the acid product of the fermentation, and under such circumstances about all that has been accomplished has been to remove one of the products from the solution. Strong alkali is very harmful to the ferment. Green found in the first work upon this castor bean ferment, which was discovered by him, that acids were inimical; hydrochloric acid, for example, in fiftieth normal solution. I have never tested the influence of this acid, but acetic acid twentieth normal is not inimical. This strength of acetic acid does not of course contain as much dissociated hydrogen as 50/N HCl. It is furthermore possible that the action is due to the molecule and is a specific chemical influence (sodium chloride is also de-

scribed as harmful) and not due to the free acidity. It would indeed be unusual if a ferment were so excessively sensitive to the presence of its natural product in the fermentative reaction. The powder of the castor bean contains over 1 per cent. of ash, including both sodium and potassium chloride, as well as the salts of the earthy metals.

An interesting instance of the conservation of a ferment by the presence of the substance related to it as the substrate is to be found in this powder. If two equal masses of the powder be exposed to high temperature in suspension in water, and to the one a fat be added, it will be found at the close of the period of heating that the powder containing the fat will have resisted destruction by heating much better than the other powder. This fact may explain why the ferment is stable so long under experimentation; it may be due to the fat present, since these are limited reactions except under certain conditions. This fact may be assumed to be the result of a combination between ferment and substrate.

Animal and vegetable lipases are able to accelerate the cleavage of the natural fats, of the esters of glycerine with the lower members of the fatty acid series, of the esters of the monatomic alcohols, of lecethins, and of the synthetic asymetric esters. In all probability the cleavage of hippuric acid and of salol, and possibly many other drugs, in the intestinal tract, is accomplished by means of lipase.

The reaction of fat fermentation. The reaction of the cleavage of an ester is an act of simple hydrolysis; the ester adds water and is then divided into an alcohol and a fatty acid. Thus aethyl acetate + water = aethyl alcohol + acetic acid. The higher fats follow the same type of reaction. Euler and Goldschmidt have attempted to interpret the reaction from the point of view of ionization. Euler found that the equilibrium of the various esters in moderately dilute solutions was not constant; the methyl esters were hydrolyzed the least, the aethyl esters the most. The hydrolysis of an ester, he determined further, was within certain limits proportional to the strength of its acid, that it is increased with the dissociation of the acid. Upon the assumption that the ester may be written $CH_3CO—O—C_2H_5$ (using

aethyl acetate for an illustration), there are two possibilities for ionic dissociation: $CH_3CO^+ — OC_2H_5^-$ and $CH_3COO^- — C_2H_5^+$. In the first case the acid would act as the base ($CH_3CO—OH$) and the alcohol as the acid ($C_2H_5O—H$); in the second case, the alcohol would act as the base ($C_2H_5 — OH$) and the acid as the acid ($CH_3OOO — H$). For this conception, which is quite analogous to the van't Hoff theory of saponification, Euler has adduced some experimental evidence. For the higher fats as well as for the simple esters it is known that an autohydrolysis occurs on solution in water. This is in all probability due to the dissociated hydrogen ion of water. Pure fats, free of bacteria, exposed to light slowly undergo cleavage into the glycerine and fatty acid. Added to this appears next an auto-oxidation, and thus the acids found following such an auto-hydrolysis are fatty acids and oxyacids. The hydrolysis is greatly accelerated by heat, and hydrolysis by the use of steam is the common commercial method of splitting fats. Hydrogen ions also act as great accelerators, and colloidal platinum has some action. Whether this cleavage occurs through the medium of intermediary reactions is not known. There is some evidence that in the cleavage of the tri-glicerides the molecules of fatty acid are split off successively. Lewkowitsch has shown that in the ordinary saponification of fats, mono- and di-glycerides may be isolated at certain stages of the reaction. In the synthesis of these fats by the aid of heat it is known that the addition of the first molecule of fatty acid may be accomplished in a relative mixture of the two components at a moderate degree of heat. For the addition of the second molecule of fatty acid, an excess of the fatty acid is necessary, and a much higher degree of heat is required. For the addition of the third molecule of fatty acid the fatty acid is no longer effective, the anhydride must be employed in excess, and the reaction is then greatly accelerated by saturation of the system with an anhydrous salt of the fatty acid; and even under these circumstances a still higher temperature is demanded. The act of cleavage thus seems to retrace the steps of the synthesis. It must not, however, be supposed that when, in a system of a soluble triglyceride as triacetin, the equilibrium is established, the cleavage be one to the diglyceride. So far as

the reaction extends, it is a complete one for the molecule; the unsplit fat is triacetin. This of course in no wise represents a contradiction of the theory of the step-like hydrolysis; this only states that the cleavage of whatever portion of the fat is split occurs in successive steps corresponding to the several molecules of fatty acid.

The equilibrium in the system alcohol + acid = ester + water is maintained only when the components are present in appropriate relations. If an excess of the alcohol or of the acid be added, all of the mass of the acid or alcohol, as the case may be, will be combined to form the ester. Thus an excess of alcohol will lead to the combination of all the acid, while upon the similar action of an excess of the acid is based the methods for the synthetic preparation of these fats. On the other hand, if an excess of water be present, the degree of hydrolysis will be increased, and if the system be sufficiently diluted with water, the ester will be nearly completely split. It is therefore necessary before employing a certain concentration of ester in digestion experiments to determine the station of equilibrium for that concentration at that temperature. This may be best accomplished by means of an appropriate acid hydrolysis, it being assumed that this catalysor does not alter the station of equilibrium. I have made these determinations for three solutions of triacetin.

The solutions were made acid to the degree of 1 mol. sulphuric acid, and the ester hydrolyzed at 20° for several months until the reaction was complete, the system stationary. For a 2 per cent solution of triacetin, the equilibrium lies at ester 26 — products 74; for a 1 per cent. solution, ester 18 — products 82; for a one-half per cent. solution, ester 12 — products 88 per cent.

Kinetics of lipolysis. The fermentation of fat presents an attractive field for the investigation of fermentation from the dynamic point of view because the hydrolysis of esters has been long a classical subject of study, representing as it does a reaction of definable and measurable reversibility. First investigated by Berthelot and St. Gilles in 1862, the reaction was recognized as one of reversible nature, terminating in an equilibrium that was approximately the same irrespective of the direction from which the reaction proceeded. The temperature had a marked

influence upon the velocity of the reaction, and some influence upon the point of equilibrium. For the different fatty acids they found that the velocity of reaction diminished with increasing molecular weight; the same rule did not hold for different alcoholds, though these gave widely varying though irregular results. Berthelot considered that the transformation in the unit of time was proportional to the mass of the reacting bodies, and inversely proportional to the volume. In their experiments they determined that the point of equilibrium of the completed reaction lay at two-thirds mol. ester to one-third mol. of acid and alcohol; the reaction could be reinaugurated by the addition of either ester or alcohol and acid.

The present formulation of the reaction is somewhat different. The equation previously given to account for the progress of a reversible reaction on the premises of the law of mass action is known not to hold experimentally for the reaction now under discussion, because it assumes that the combination of fatty acid and alcohol in the one direction and the cleavage of the ester in the other direction proceeds at a rate dependent solely upon their several masses, and upon the dissociated hydrogen of the solvent water; this is not true, because one of the components, acetic acid, acts as an auto-catalysor. The acetic acid is in part dissociated, and the dissociated acid acts as a positive catalysor. When alcohol and the fatty acid are mixed, the catalysor is present in greatest amount at a time when the active masses are greatest, therefore the acceleration in the direction of combination is marked; when the ester and water are mixed, the autocatalysor is not present, and appears only very gradually, is greatest when the active masses are small, therefore the acceleration is not pronounced in the direction of cleavage. As the reaction alcohol + fatty acid proceeds the catalysor is gradually reduced, while in the other reaction it is gradually increased. When the station of equilibrium is reached, it is the same in either case, but it requires a longer time in the case of the reaction ester + water than is the case of the reaction alcohol + fatty acid. Knoblauch has shown that when hydrochloric acid is added in such quantity to each system as to make negligible the influence of the dissociated acetic acid (thus maintaining throughout a constancy of the hy-

drogen ions) the velocity constants of the opposing reactions are equal.

Kastle and Loevenhart have studied the kinetics of the fermentation of ethyl butyrate by animal lipase. They found the reaction usually incomplete; if the ferment were very active or the substrate concentration very low, a practically complete reaction was sometimes attained. The constants of velocity diminished through the course of an experiment. This velocity was not directly proportional to the active mass of the substrate. With constant substrate concentrations, the velocity was directly proportional to the quantity of ferment. They found the optimal temperature at 40°. More recently Kastle has again studied the kinetic relations. He found that the animal lipase employed was completely retained by a porcelain filter. He demonstrated that the reaction was monomolecular, and that in general the results followed the equation for a reaction of that order, though there was some lagging which was attributed to the acid set free. The ferment was not appreciably destroyed in the reaction. The transformation was not directly proportional to the substrate concentration. He also determined the interesting fact that while the lipase in watery solution was soon destroyed, it would keep for a long time in a very weak acid solution.

Volhard[2] and Stade, working with gastric lipase and yolk of egg, found the velocity of transformation proportional to the substrate concentration. Their figures are, however, only rough, their method cannot be considered accurate.

There are theoretical considerations that indicate the impossibility of securing quantitative results in the fermentation of natural fats, and these are based on the inability to control the substrate concentration. When we speak of a fluid fat, we mean a fat in which at the temperature present the triolein is able to hold the tripalmitine and tristearine in solution. When we speak of a congealed fat, we mean a fat in which at the temperature present the triolein is not able to hold the other two fats in solution. The triolein is a solvent for the other two fats. The so-called melting point of a natural fat is not a physical melting point at all, but is the temperature at which the triolein in a particular fat is able to dissolve the tripalmitine and tristearine. The

oelic acid has the same relations of solubility for both the palmitic and stearic acids and fats. Now in a fermentation experiment, which fat is first attacked? We do not know, though there is some evidence that tristearine is most easily split. But whichever were first hydrolyzed, it is clear that the substrate concentration could not be constant through the experiment, and all the more so when it is realized that one of the products of the reaction (oleic acid) is a solvent for the substrate. The relative insolubility of natural fats is a great drawback. If one were to attempt to operate with fluid fats and ferment alone, the reaction (which does occur) would be a bimolecular one, and for these we have for the substances and circumstances under consideration no mathematical development.

Since one ought to work with a known fat, which shall it be? The lower fats of the saturated series are soluble, and in every way adapted to the needs of the experiments. As a rule the fats of the mon-atomic alcohols have been employed, such as ethyl buterate. To the use of these simple esters there are many objections. They are volatile, and the substrate concentration is hard to control. They are unstable. They exhibit the reversed reaction (the formation of the ester from the alcohol and acid) to a measurable degree. The cleavage is measurably accelerated by acids, and thus the one product (the fatty acid) acts as an autocatalysor. Lastly they exhibit a much greater chemical resistance to ferments than do the esters of glycerine; and this resistance is in marked contrast to the greater readiness of their hydrolysis by acids, to which the glycerine esters are highly resistant.

I have employed in my studies the triglyceride of acetic acid. This may be prepared upon a large scale in a state of almost absolute purity, free of water, and of acidity. It is scarcely volatile, does not disintegrate, the auto-hydrolysis does not occur with measurable rapidity in watery solutions, the reversion does not occur at ordinary temperatures with measurable rapidity, the substance is resistant to acids, and thus auto-catalysis by the product, acetic acid, is slight. To illustrate the pronounced character of these qualities, figures may be given. Adequate fermentation tests with this ester may be completed within a hundred hours, at room temperature. At this temperature no reversion

of the reaction, no formation of ester from a mixture of glacial acetic acid and water-free glycerine, can be obtained in less than a couple of months. In the concentration of substrate employed, no acidity (auto-hydrolysis of the ester) will appear at the temperature employed within a month. The addition of that degree of acidity that could be developed at the completion of the fermentation will not at the temperature used split a measurable amount of the ester within a week. This ester is very susceptible of fermentation, particularly with the lipase of the castor bean, with which I have most experience. These qualities make work with this substrate a real pleasure. The measurements are accurately accomplished by a simple titration. As against the higher members of the same series, this fat has the great advantage of forming a true homogeneous solution.

In carrying out experiments with the castor bean powder it is necessary to modify somewhat the usual method of procedure. In an ordinary experiment of catalysos in a homogeneous system, and in such fermentations as present a high degree of intermixture, it is assumed that all parts of the mass have the same composition. This is true for the homogeneous system; it is so nearly true for an ordinary colloidal mixture that the error may be neglected. But in systems in which a veritable gross suspension and emulsion exist, such an assumption may no longer be made. It is not under these circumstances permissible to prepare a large volume of the system under investigation and from time to time remove a portion and determine the degree of disappearance of the substrate or the appearance of the products. One is compelled to prepare many tests, often as many as thirty or more, and employ one for each quantitative determination. This procedure exposes the results to the error that is certain to follow the slight variations in the preparation of the individual tests—the errors in the different weighings, in the titrations, and also the error in the assumption that each gram of the powder has the same activity. These errors may be minimized by a very careful mixing of the powder, accurate weighing, etc., but they cannot be removed. Proper mixture of the tests means also proper and continued shaking. This is less important in fermentations of soluble than of suspended fats. A lack of shaking re-

sults in a retardation of the reaction. Fortunately the fat reactions are quite slow, so that the factor of stirring is less imperative than in very rapid reactions. It is best to employ a mechanical shaking apparatus.

The triacetin was prepared according to the method of Seegen, and was rectified twice by distillation at 174° at 25 mm. pressure. It was colorless, neutral, and entirely soluble in fifty parts of water.

The temperature varied within a degree of 18°; we had no thermostat large enough to accommodate the numerous flasks. The error was not serious.

Employing triacetin as the substrate, I have found that the rate of transformation follows very closely the logarythmal curve. Using solutions of one-half, 1 and 2 per cent. strength, with one gramme of ferment in the vol. of 100 c.c., at 18°, the following constants were obtained, calculated by the equation $K = \frac{1}{t} \log \frac{A}{A-x}$ the constants are:

t=hours.		4	8	16	24	28	32	40	48
½%.	$\frac{x}{A}$	0.096	0.162	0.287	0.418	0.489	0.477	0.623	0.652
	C	109	96	92	98	104	88	106	96
1 %.	$\frac{x}{A}$	0.083	0.174	0.338	0.418	0.488	0.542	0.609	0.655
	C	94	104	112	98	104	106	102	96
2 %.	$\frac{x}{A}$	0.098	0.174	0.323	0.431	0.502	0.485	0.595	0.636
	C	112	104	106	102	108	90	98	91

Despite the fluctuations, these figures are quite regular when all the conditions of the experiment are taken into consideration. They may be justly said to indicate that the transformation in the unit of time is directly proportional to the active mass of the substrate. Apart from the slight irregularities, these figures for the constants for three different concentrations of substrate are closely enough concordant to give the constants a real value, such a value as they should have according to the equations. This stability of the figures for the constants is reinforced by another

fact, the observation that the same stations of equilibrium observed for these concentrations with the use of sulphuric acid as the catalysor, are closely reproduced by these fermentations.

	½%	1%	2%
Acid	12/88	18—82	24—76
Ferment	14—86	21—79	30—70

This result shows further that the ferment does not participate in the reaction in any other capacity than as a catalysor, which we have seen is not true of other ferments as a rule.

If we apply to these results the equation of Henri $-\frac{dx}{dt}=(\frac{C\ (A-x)}{1+m(A-x)+nx})$ it follows that m and n are equal.

Nicloux investigated the cleavage of olive oil by the castor bean enzyme. He found that when the ferment was present in considerable amount, the constants calculated for the reaction in a monomolecular reaction were quite constant.

Stade has studied the fermentation of the fat in the yolk of egg by gastric lipase. The constants, as calculated from his results by Euler, display a rapid and progressive diminution, which was ascribed to the influence of the fatty acid.

Other studies have been carried out upon emulsified fats by Fromme, Engel, Henri and Nicloux, Zellner, and Kanitz. Nicloux and Henri and Nicloux found the transformation roughly proportional to the time, though with a higher concentration of substrate there was some relation to the substrate mass. Zellner found the transformation constant in the unit of time, while Kanitz found the transformation roughly proportional to the square root of the time. The experiments of Volhard, Stade, Fromme, Engel, as well as of the earlier experiments of Connstein, Hoyer, and Warteburg, were not carried out from the point of view of the law of mass action, and a recalculation of their data gives irregular results.

I have further studied the hydrolysis of aethyl acetate, and here the curious observation was made that this ester is much more resistant to this ferment than is triacetin. For ordinary acid hydrolysis or saponification, aethyl acetate is more easily hydrolyzed than triacetin, but with this ferment the relations are reversed. Not only is aethyl acetate resistant to the ferment,

but the results are so irregular as to be of little value. This relation to these two esters is also true of pancreatic lipase; it digests triacetin much more easily than aethyl acetate.

Very interesting have been the results with triolein. The triolein was prepared by fractional saponification of olive oil in the cold, extraction with aether, washing with sodium carbonate, and purified by several times being passed through acetone and aether. It was neutral, of a pale yellow color, odorless, and entirely soluble in water-free aether. It gave the iodine number 85, the saponification number 191. This was employed in 2 per cent. suspension, with 1 per cent. suspension of the ferment powder. The results for the observations is as follows; t is days.

c.c. 10/ normal acid. tempt. 18.0.

1	2	3	4	5	7	9	11	16	18
3	5	7.5	9	11	16	21.6	27.2	32.1	38.1

These give the following increases in acidity for each day.

3 — 2 — 2.5 — 1.5 — 2 — 2.5 — 2.5 — 2.8 — 2.8 — 2.8 — 2.8 — 2.2 — 2.2 — 2.2 — 2.7 — 3.7 —

Though it must be stated that there is quite an error in the method of estimation—an extraction with aether followed by the titration of the aether alcoholic solution of the oleic acid—it is apparent that the rate of transformation is a function of the time. A somewhat similar result has been reported by Henri and Nicloux. They found that for the ferment of the castor bean there was an optimum concentration of acid. When at the largest optimum concentration of acid they fermented different concentrations of oil, varying from 25 to 87 units, the transformations in all were approximately identical in the unit of time. Their ferment was much stronger than any employed by me.

The system triolein-lipase-water is a two-phase system in the strict sense of the term. The lipase and triolein are almost insoluble in water. It may in all probability be assumed that the triolein and the lipase are in a colloidal complex; the minute traces in solution may be assumed to be in a complex chemical combination. When the first fractions of the triolein have been hydrolyzed, the one product, glycerine, passes into the water phase, in all probability quite completely. The other product, oleic acid, may be assumed to be divided between the water phase

and the colloidal phase, in large part in the latter. That fraction of the oleic acid present in the water will render the triolein more soluble than in water alone. The reaction may be assumed to occur on the boundary film of the colloidal complex. As the reaction proceeds, the dimension of this boundary film is diminished. Under these circumstances, the experimental finding—that the transformation is a linear function of time—is best explained by the Nernst hypothesis that the experimental velocity is a diffusion velocity. That the experimental findings do not correspond well with the theory of reaction in a homogeneous system is indicated also by a further consideration of the conditions in the experiment.

Triolein, glyceride of oleinic acid, is almost insoluble in water; it is so little soluble that a saturated solution at ordinary temperature could not be employed for even a qualitative study. Oleic acid is very insoluble; it is so insoluble and so little dissociated that a saturated solution in water at ordinary temperature scarcely conducts the current better than the water with which the solution has been prepared. But the saturated solution of oleic acid is a much better solvent for the triolein than is the water; and analogously a solution saturated with triolein and oleic acid is the best watery solvent for the other higher fats. Now, discarding for the moment the solvent action of the fatty acid, an experiment with the hydrolysis of triolein would always present a saturated solution of the substrate; if one began with sufficient triolein to actually accomplish an experiment of value, one would begin with a suspension, and as in each moment a certain amount of the ester would be hydrolyzed, its place in the solution would be taken by a new portion that had passed into solution. Therefore the substrate concentration would through the greater part of the experiment until almost the end remain constant, and according to the law of mass action, the transformation in the unit of time would remain constant. Since the solubility of the one product, oleic acid, is also very low, this would soon begin to be thrown out of solution. Thus we would have the condition of a catalytic reaction in a system saturated with the substrate and saturated with one of the products—a most anomalous condition. This is what actually

occurs in an acid catalysis of a fat; in each moment fatty acid is being thrown out of solution, and in each corresponding moment a fraction of fat is dissolving; and whereas in the beginning there was a heterogeneity in the system due to the substrate (the fat), at the close of the reaction there remains a heterogeneity in the system due to the one product, the fatty acid. Under these circumstances, the reaction becomes practically a complete cleavage, and does not display a point of equilibrium such as is usually seen in the hydrolysis of an ester. In the film of contact between the phase of the solvent and of the suspended fat, the substrate is in hypersaturated solution; and as the reaction there occurs with rapidity, there must exist in this film also supersaturation of the fatty acid. On stirring the mixture, as this fatty acid diffuses from the film of contact it will pass out of solution. The process continuing in this manner, the reaction would proceed until the fat had all passed into solution, and the velocity of the reaction would depend solely upon the rate of diffusion. These reactions would be in part altered by the effect of the oleic acid upon the solubility of the fat, since in this behavior the oleic acid plays the rôle of a solvent, and thus alters the concentration of the substrate and increases its active mass. This in itself might be of little importance, since the reaction in the film may be so rapid as to be independent of the substrate concentration in the sense of the law of mass action. But it might be of importance if the rate of diffusion of the bodies were altered by the presence of the greater content of fat and fatty acid in the solvent. In all likelihood, the rate of diffusion is somewhat altered; but this is probably of no practical account, because the rate of diffusion of fats and fatty acids of low solubilities is exceedingly slow. It is, however, probable that in this case the increase of the substrate brought about by the fatty acid might lead to an appreciable increase in the velocity of reaction at the film of contact, because the unaided solubility of the fat would be so extremely low. In any event, it is not probable that the alterations effected by the oleic acid would determine notable quantitative disturbances, because though it is assumed in the Nernst theory that the substrate must be so slightly soluble that it does not participate appreciably in the diffusion, even with the aid of the oleic

acid, the solubility of the fat is so slight as to lie well within this reservation. I cannot see how the Nernst theory does not in every way correspond to the conditions in an acid hydrolysis of a higher fat. In the earlier consideration of this theory I stated that for many fermentations the heterogeneity of the system was often so slight that it was doubtful whether the conditions of the theory were adequately fulfilled; but in the case of the higher natural fats the system is typically heterogeneous, as typically so as in the reactions studied by Brunner under the direction of Nernst. It is true that the reaction velocity is a function of time in a reaction in a homogeneous system where the active substrate is constant, the condition postulated above for this system. But since the application of this point of view to reactions in a heterogeneous system make the velocity also a function of the dimensions of the boundary of contact of the two phases, the theory would not fit the facts here because while the transformation is uniform the dimensions are constantly decreasing. The oleic acid is passing out of solution, it is true, but the reactions cannot be supposed to occur at the boundary of its particles.

The substrate is a suspended particle, very slightly soluble in the medium (though this solubility will be somewhat increased by the presence of one of the products), and this substrate diffuses very slowly; the products are very slightly soluble in the medium (the fatty acid at least; the alcohol is soluble) and diffuses very slowly; and the catalysor is a suspended particle, and the reaction may be assumed to occur in large part or wholly in the film of contact of the catalysor and substrate. Obviously there are two streams of diffusion: the one connected with the solution of the substrate, and the second connected with the reaction at the film of contact of the solution and the catalysor. It seems certain that when one considers the facts—considers the prolonged constancy of the active mass of the substrate from the point of view of the law of mass action, the known rapidity of the reaction in the film of contact of two phases, the demonstrable slowness of the diffusion, and finally the necessity of two diffusions, one must believe that here certainly the theory of Nernst does apply with full force, and that in accordance therewith the so-called reaction velocity of a fat fermentation with

the use of fats of the higher series in reality represents a diffusion velocity solely. As Heimbrodt has shown, under proper conditions of concentration, the formulation of the theory leads to an equation quite identical with that corresponding to a monomolecular reaction in a homogeneous system.

This interpretation of the phenomenon is supported also by the observations on the effect of increase in temperature on the fermentation. An increase of 10° does not double the velocity; it increases it not over one-fourth, just such an increment as would be expected from the known influence of increase of temperature on diffusion velocity. Senter, in a recent paper, states that the same relation to temperature holds for the reduction of hydrogen peroxide by hamase, the blood peroxidase, the increase of velocity with increase in temperature, were not what would be expected in a chemical reaction, but corresponded well to what would be expected in a diffusion experiment.

The point of view of Herzog cannot be applied to this digestion at all because the viscosity of the system is practically unchanged throughout the experiment. The emulsion triolein-ferment powder-water has the same viscosity as the emulsion oleinic acid-glycerine-ferment powder-water, within the range of concentrations at least that are involved in these experiments.

Relation of acceleration to mass of ferment. The acceleration of the hydrolysis of triacetin has been found directly proportional to the ferment present. This is illustrated in the following experiment.

Substrate constant: (*a*) ferment 2 per cent; (*b*) ferment 1 per cent. The times are those necessary for the proportional transformation of the stated units of acid.

units	10	20	30
a time	23	51	104
b time	47	97	216

Kastle and Loevenhart, working with animal lipase and aethyl butyrate and monobutyrin, found the acceleration proportional to the mass of ferment. Vollhard and Stade, working with gastric lipase and the yolk of egg, found the acceleration roughly proportional to the square root of the ferment. It is clear, however, that the yolk of egg method cannot be used for

quantitative study. Engel also found the Schutz rule to hold for lipase, and this has been stated further by members of the Pawlow school, and also by Benach and Gayot. Engel compared the times necessary to split off 1 per cent. of acid; the Pawlow procedure, on the contrary, is to compare the work done in a fixed time—a theoretically improper procedure.

The reversion of the fermentation. This has been repeatedly accomplished, first by Kastle and Loevenhart and by Bernizone for aethyl buterate, by Hanriot[2] for monobutryin, both employing animal lipase. In a previous publication I reported upon the synthesis of triolein through the activity of the castor bean ferment, and upon the failure of the synthesis of the same fat by pancreatic lipase, the latter experience being but the repetition of the previous failures of other investigators.

Pottevin has recently described the synthesis of numerous esters, including those of oleinic and stearic acid, by pancreatic powders, operating in the absence of water. The report states that the water-free fatty acids are suspended in the alcohol and the dry powder added, and that this powder does not go into solution. Theoretically the synthesis ought to occur with rapidity, since it must be assumed to occur at the boundary of contact of the ferment phase. The conclusion of the author, that the pancreatic lipase acts as a hydrolytic ferment in watery solution, and as a combining ferment in the absence of water, is not supported by the experimental evidence, and may be rejected on theoretical grounds. If enzymic fat synthesis occur at all in natural life, it occurs always in the presence of water. I have, however, been able to confirm the Pottevin statement for triolein.

Theoretically the acetic acid would act as an auto-catalysor, but practically no such action is to be discerned in the constants. It might be supposed that possibly an auto-catalysis just covered the loss of the ferment, and thus hid itself; but direct experiments to this end indicate that no material inactivation of the ferment occurs in an experiment of such length. The equilibrium may be brought about by the addition of the theoretical amount of acetic acid (the alcohol has little effect); the neutralization of the acetic acid will reëstablish the reaction in a system in equilibrium, as will the addition of more substrate. The addi-

tion of more ferment, however, will not reinaugurate the reaction in a system in equilibrium. If one mix the acid alcohol and ester in the proportions determined to constitute the equilibrium, the addition of ferment will not result in any alteration of these relations.

Conditions of maximum activity. For animal lipase the statements concerning the favorable reaction are contradictory. A faint alkalinity is stated by some to be most favorable, by others a faint acidity. Obviously a faint alkalinity soon means neutrality in the experiment, since the fatty acid set free in the reaction would neutralize the reaction. Animal lipase is so sensitive and is in such a mixture of unstable substances that it seems to vary widely with each different preparation. In the body it acts in a neutral reaction. In a test with the higher fats, the products of the reaction could never give rise to a marked acidity. The vegetable lipases are very stable substances under favorable circumstances. Alkali is deleterious, the fatty acids not at all so in moderate concentrations. The heavy metal salts kill the castor bean lipase rapidly, but the ordinary electrolytic salts (the chlorides and sulphates of potassium, sodium magnesium, and calcium) have no effect at low concentrations. Vegetable lipase is resistant to digestion, particularly to pepsin. The results with trypsin are complicated by the vulnerability to alkali. The powdered castor bean contains a proteolytic ferment, which, however, seems less capable of attacking it than does mammalian trypsin. Purification of this ferment is a waste of time; the fat-free powdered beans give the best results so far as the integrity of the ferment is concerned. Of zymoexciters I have found none. Bile and lecethin, that are zymoexciters of animal lipase, do not act so for the castor bean lipase, rather the contrary. Extracts of liver or other tissue do not enhance the action of this lipase, nor do extracts of the leaves and stalks of the castor plant.

According to Hewlitt, lecethin acts as a powerful zymoexciter to pancreatic lipase. It is without action on vegetable lipase. Magnus has described a zymoexciter that exists in the liver; it is a diffusible substance, is not soluble in aether, and is not destroyed by boiling. According to Hoyer, mangano salts act as zymoexciters to vegetable lipase.

Chemical properties of ferment. Lipase has not been isolated in even the rough form that has been accomplished for other ferments. It is retained to some extent by filtration through paper, to a greater extent by filtration through infusorial and porcelain filters; it has no power of dyalisis. The ferment seems quite insoluble in the true sense; the suspensions are always very colloidal. All preparations that I have seen gave typical reactions for protein. The suggestion of Hanriot that lipase might be an iron-organic complex is devoid of chemical support. On suspension in water in the absence of an ester, lipase is rather rapidly destroyed. This destruction seems to be a simple hydrolysis, and the velocity is in general proportional to the mass and increases with increase in temperature. The products of the fermentation of the natural facts act in a protective manner, although to less extent than do the fats. The esters of the common alcohols do not protect lipase from hydrolysis as do glycerine fats, while the products are directly injurious. This action is the property of the alcohol; lipase is sensitive to aethyl alcohol in low concentration.

LITERATURE.

Volhard.[1] Zeitschr. f. klin. Med., *43*, 397.
Benech and Guyot. C. r. Soc. Biol., *55*, 719, 721.
Hanriot.[1] C. r. Soc. Biol., *48*, 925.
C. r. Acad. Sc., *123*, 753.
Euler. Zeitschr. f. physik. Chem., *36*, 405.
Knoblauch. Zeitschr. f. physik. Chem., *22*, 268.
Kastle and Loevenhart. Am. Chem. Jour., *24*, 491.
Kastle, Johnson and Elove. Am. Chem. Jour., *31*, 521.
Volhard.[2] Zeitschr. f. klin. Med., *42*, 414; *43*, 397.
Volhard. Zeitschr. f. klin. Med., *42*, 414; *43*, 397.
Stade. Beitr. z. chem. Physiol. u. Path., *3*, 291.
Seelig. Berichte, *24*, 3466.
Nicloux. C. r. Soc. biol., *56*, 840.
Fromme. Beitr. z. chem. Physiol. u. Path., *7*, 51.
Engel. Beitr. z. chem. Physiol. u. Path., *7*, 77.
Henri and Nicloux. C. r. Soc. Biol., *57*, 175.
Zellner. Monatsch. f. Chem., *26*, 727.
Kanitz. Zritschr. f. physiol. Chem., *46*, 482.
Connstein, Hoyer, and Warteburg. Berichte, *35*, 3988.
Heimbrodt. Ann. d. Physik. (IV), *13*, 1028.
Bernizone. Atti del. Soc. Lig. di. Scien. nat. e Geograf. Genoa, *11*, 327.
Hanriot.[2] C. r. Soc. biol., *53*, 70.
Pottevin. C. r. Acad. Sc., *138*, 378.
Taylor. Univ. Cal. Pub. Pathology, *1*, 33.
Hewlitt. Jour. Med. Res., *11*, 377.

THE NATURE OF FERMENTS.

THE REACTION OF FERMENTATIONS.

We know very little of the chemical nature of ferments. They are always associated with numerous bodies derived from the cells to which they owe their origin, and they are so labile that isolation is attended with denaturation and decomposition. We may say that ferments are proteins, or closely resemble them. This general conclusion is based upon the facts that ferments are usually coagulable, they respond to the color tests and reactions for protein, they are precipitated by the ordinary salts, they yield on hydrolysis or digestion, to which they are all more or less susceptible, amido acids. These observances are, however, alike in the case of no two ferments. Their physical properties likewise vary. Some are very colloidal, others diffuse. Some rotate light, and are thus known to contain asymmetrical carbon; others are optically inactive. Some contain a carbohydrate moiety, and thus appear to resemble glycoproteids. Others contain a large amount of phosphorus in organic combination, and on digestion yield purin bodies, and thus appear to resemble neucleoproteids. In fact the best studied of the animal ferments, pepsin, exhibits these qualities. Other ferments contain no neuclein or carbohydrate, are not coagulable, and resemble proteose. Thus only the most tentative opinions may be passed upon the chemical nature of these bodies. Loew and his students, despairing of a general characterization of the nature of ferments, have attempted to define the active groups of the molecule. They assume that ferments are able to accelerate the velocity of reactions by the possession of active labile groups. And these Loew considers to be in all probability keton and amino groups. These conclusions are based upon the experimental study of such chemical procedures whose reactions may be assumed to indicate such groups; thus the action of hydrazine, cyanogens, and methylamine indicate the presence of keton groups; the presence of amino groups is indicated by the reactions with nitrous acid and

dicyanogen. It is doubtful whether these considerations are of general application. Pollok has apparently shown that by the action of acid and alkali on trypsine the ferment may be robbed of its power of digesting albumine and globuline and fibrin, without any loss in the faculty of digesting gelatine. Without assuming that trypsine is a mixture of ferments, each more or less specific for particular proteins, he suggests that these different functions are associated with different group radicals of the molecule.

The chemical nature of fermentation. A discussion of the chemical nature of the fermentative acceleration must in the nature of the phenomenon be based primarily upon a study of the nature of catalytic accelerations in general, and upon the demonstration of analogies and reactional relations existing between them. Since it seems certain from the thermodynamic point of view that a catalysor or ferment acts only by the direct or indirect reduction of the internal resistance, and not by any increase in the driving force of the reaction, all investigations must be directed to the internal physical and chemical resistance.

In the consideration of the modus operandi of the catalytic acceleration, we thus face directly the question of the nature of the internal resistance to chemical reactions that is a property of all substances. Since a catalytic acceleration is defined as an acceleration due to the lowering of the internal resistance of the substance, we must attempt to define a conception of chemical resistance. There has been little study of this aspect of the question. Of the two factors in every reaction—the driving force and the internal resistance—nearly all the physico-chemical research has been directed to the driving force. That the constitution of organic molecules is associated with variations in the resistance to reactions is of course well known. Examples of such relations will be given in the chapter on specificity of ferment action. But the same factor of resistance resides in the most simple inorganic substance. In any event, it is certain that the term internal resistance stands not for one thing, but may stand for many things that are varied from case to case. Under these circumstances, therefore, the means whereby a catalytic agent lowers this resistance may vary from case to case, and must be studied anew for

each individual reaction. One of the future achievements in chemistry will be to define an Ohm's law for chemical reactions.

There are several ways in which the presence of a catalysor might accelerate the velocity of a reaction. Firstly it might reduce the chemical resistance of a substance, just as temperature does; it might operate against some of the inhibitory influences of which van't Hoff speaks. Secondly, it might reduce the number of intermediate reactions that occur naturally in the reaction. An illustration of this in the domain of inorganic chemistry is to be seen in the action of a cobalt salt on the reaction between NaOH and Cl. This would amount to a short cut to the final product, and if the velocity of each step were no greater than in the ordinary reaction, the total velocity would be greater. Thirdly, new intermediary reactions might be introduced, of greater velocity, so that the sum of their velocities would be greater than the velocity of the original reaction. A quantitative illustration of this is to be found in the acceleration of the reduction of hydrogen peroxide by hydriodic acid under the catalytic influence of molybdic acid.

The theory of intermediary reactions is not only the oldest theory, having been suggested by Clement and Desormes, Mitscherlich, Traube, Schoenbein, and de la Rive, but it has in its favor a large amount of experimental evidence by Engler, Woehler, Wagner, Haber, Brode, Kastle and Loevenhart, Luther, Schilow, Bach and Cordat, and others. The theory is in brief that a catalysor accelerates the velocity of a reaction by the introduction of intermediary reactions with the formation of unstable products, usually of the type of addition-products; that these products are themselves so unstable that they disintegrate of their own accord or they are disintegrated by the action of other bodies in the system; and that the sum of the velocities of the several reactions is greater than the velocity of the primary reaction. Wegscheider[1] further separates simple accelerations (Folgewirkungen) from accessory accelerations (Nebenwirkungen), depending upon whether the end-product of the accelerated reaction is the same as in the unaccelerated reaction. The theory has been tested solely upon catalyses of pure reactions, usually of inorganic nature. Thus far the evidence is almost

entirely qualitative; in the work of Brode upon the acceleration of the reaction between hydrogen peroxide and hydriodic acid by molybdic acid, the kinetic relations were worked out in such a manner as to demonstrate that the sum of the velocities of the several reactions was greater than the velocity of the natural reaction.

It is usually assumed that even the simplest reactions are not accomplished directly; they, too, pass through intermediary stages (Wegscheider); and thus the catalysor by the introduction of other intermediary stages does not in the least alter the general nature of the proccss of reaction, but by introducing intermediary reactions with less of chemical resistance effects a short cut to the stage of equilibrium. Experimental researches indicate that while in perhaps the majority of these accelerations the number of intermediary reactions is increased, instances are known in which the number of intermediary reactions has been diminished. In any event, be the number in the catalytic series greater or less, the theory assumes that the sum of their velocities is greater than was the velocity of the original reaction. Obviously the theory can be properly tested only upon reaction for which we can determine the intermediary reactions with and without the catalysor. These intermediary reactions are to be studied from the standpoint of the Ostwald law of reaction-stages: In all chemical reactions the most stable condition is not attained at once, but either the nearest reaction is attained or among several possible reactions the most unstable, etc., etc. Thus $Cu\ SO_4 = 2\ KOH = K_2SO_4 + CuO + H_2O$ passes through one intermediary stage.

$$CuSO_4 + 2\ KOH = K_2SO_4 + Cu(OH)^2.$$
$$Cu(OH)_2 = CuO + H_2O.$$

Now when to such a system an appropriate positive catalysor is added, new intermediary stages are introduced, termed for a certain large class of reactions stages of primary oxides; the primary oxides have some of the behaviors of peroxides; they are stronger oxidizors than the highest stable oxidation-stage and stronger reducers than the lowest oxidation-stage (Luther).

$$2\ H_2O_2 = 2\ H_2O + O_2.$$

In the presence of ferric oxide this reaction is very rapid.

$$2\ H_2O_2 + Fe_2O_3 = \text{iron primary oxide } (Fe_2O_3.O_2) + 2\ H_2O.$$
$$Fe_2O_3.O_2 = Fe_2O_3 + O_2.$$

A reaction in more stages is as follows:

$$4\ NaOH + 2\ Cl_2 = 4\ NaCl + 2\ H_2O + O_2.$$

$$24\ NaOH + 12\ Cl_2 = 12\ H_2O + 12\ NaCl + 12\ NaClO.$$
$$12\ NaClO = 8\ NaCl + 4\ NaClO_3.$$
$$4\ NaClO_3 = NaCl + 3\ NaClO_4.$$
$$3\ NaClO_4 = 3\ NaCl + 6\ O_2.$$

Here there are three stages of primary oxides, and each more "primary" than the succeeding one. When a cobalt salt is added the reaction is accelerated.

$$24\ NaOH + 12\ Cl_2 = 12\ NaCl + 12\ H_2O + 12\ NaClO.$$
$$12\ NaClO + \text{cobalt salt} = 12\ NaCl + \text{cobalt primary oxide.}$$
$$\text{Cobalt primary oxide} = \text{cobalt salt} + 6\ O_2.$$

Here all the NaCl are formed in the first and second stages; that is, the number of intermediary reactions is reduced.

The reaction of potassium permanganate with hydrochloric acid has its direct expression in the following formula:

$$KMnO_4 + 8\ HCl = KCl + MnCl_2 + 4\ H_2O + 5\ Cl.$$

Platinic chloride accelerates this reaction in the following way:

$$KMnO_4 + 4\ H_2PtCl_6 = KCl + MnCl_2 + 4\ PtCl_4 + 4\ H_2O + 5\ Cl.$$

the platinic chloride having combined with the hydrochloric acid to form the chloroplatinum acid H_2PtCl_6, which is more rapidly reacted upon by permanganate than is hydrochloric acid.

The reaction between hydrogen peroxide and hydriodic acid is expressed in the following equation:

$$H_2O_2 + 2\ HI = 2\ H_2O + I_2.$$

$$H_2O_2 + I^- = H_2O + OI^-.$$
$$OI^- + 2\ H^+ + I^- = H_2O + I_2.$$

When molybdic acid is added to the system we have, expressed in its simplest terms:

$$H_2O_2 + H_2MO_4 = H_4MO_6.$$
$$H_4MO_6 + 2\ HI = H_2MO_4 + 2\ H_2O + I_2.$$

The sum of the velocities of these reactions is very much greater than in the original reaction. (Brode.)

Kastle and Loevenhart have recently discussed the action of hydrogen peroxide and have given strong evidence for the older view that whenever hydrogen peroxide acts as an oxidizing or reducing agent it first combines with the substance to form an unstable peroxide-like body.

Thus $H_2SO_3 + H_2O_2 = H_4SO_5$.
$H_4SO_5 = H_2SO_4 + H_2O$.
And $Ag_2O + 3\ H_2O_2 = H_4Ag_2O_6 + H_2O$.
$H_4Ag_2O_6 = 2\ Ag + 2\ H_2O + 2\ O_2$.

Closely related to the theory of intermediary reaction is the Kessler theory of induction. When two reactions are going on in the same solution, the presence of one may accelerate the velocity of the other. On close analysis it is seen that the actual process is one of intermediary reaction. All instances of reaction by induction are of course not catalytic, but many of them cannot be otherwise defined on account of the existence of a slow primary reaction. Theoretically, induced reactions may be divided into two groups: those in which the intermediary products are stable, and those in which they are labile. In catalyses we have apparently to deal with those in which the intermediary reactions are labile. The two bodies in the reaction whose presence results in the acceleration (the primary, voluntary reaction) are termed the inductor and the actor, while the body whose reaction is induced to an acceleration is termed the acceptor, the actor being the same in each reaction. The intermediary body may be either a combination of the actor with the inductor, or it may be an unstable addition product of the actor or of the inductor. Good illustrations may be given from the group of oxidations, though the phenomenon is not at all confined to oxidations; it is, on the contrary, in all probability a phenomenon of widespread occurrence and fundamental importance. The simplest type is where the acceptor D is slowly reacting with the actor A; when the inductor I is added it also reacts with the actor A, and then the product reacts with the acceptor D, as a result of which the actor A associated with I is transferred to D; that is, I induces more of A to react with D than before.

$D + A = DA$ — very slow.
$I + A = IA$ — very rapid; then
$IA + D = I + DA$,

so that the velocity of the formation of DA is increased.

The reaction $SO_2 + O = SO_3$ is very slow. When in the system, however, the reaction ferrous salt + oxygen = ferric salt is going on, the combination of the sulphur dioxide is greatly accelerated. The reaction between the iron salts acts as the carrier of oxygen; as fast as the ferric oxide is formed it is reduced by the sulphur dioxide.

$SO_2 + O = SO_3$ — slow.
$2\ FeO + O_2 = 2\ FeO_2$ (possible Fe_2O_5) rapid; then
$So_2 + FeO_2 = SO_3 + FeO$.

The reaction from the ferrous to the ferric salt must be kept going by an appropriate catalysor. This method now constitutes a commercial method for the manufacture of sulphuric acid. Many of the induced reactions are not so simple, in that there is no reaction between the product of the second reaction and the acceptor. For example, chromic acid, or its salts, is not able to oxidize tartaric acid with measurable velocity; it oxidizes arsenious oxide with great rapidity. When the two reactions are accomplished in the same system the tartaric acid is also oxidized.

Chromic acid + tartaric acid = formic acid and other acids — very slow.
Chromic acid + arsenious acid = arsenic acid — very rapid.
Chromic acid + tartaric acid = formic acid — rapid.

Here the product of the primary reaction, arsenic acid, is stable; when the reaction is completed the arsenious acid is entirely oxidized, the tartaric acid in large part. The relations have therefore not been those of an oxygen carrier, as in the first illustration. The explanation is that some intermediary stage in the reaction chromic acid + arsenious acid provides the point of departure for the impetus of the second reaction. This may be represented as follows:

Chromic acid + arsenious acid = intermediary product = arsenic acid.
Intermediary product + tartaric acid = formic acid, etc.

Another illustration is furnished by bromic acid, which does not act upon arsenious acid, but reacts rapidly with sulphurous acid;

when the reactions are associated, the arsenious acid is also oxidized.

As the subject of inductions is investigated, it becomes apparent that many of the accelerations by intermediary reactions are of this nature. All the activations of oxygen, in which the formation of peroxide-like bodies is probable, belong to the simpler reactions by induction. Indeed the coupled reactions should be a sub-class of the transformations by intermediary reactions. It will, on the contrary, not be possible to class all the catalyses as induced reaction, numerous and important as these certainly are. Luther and Schilow have recently studied these inductions, and believe that the process follows one of two relations, depending upon whether the intermediary stage is stabile or labile. And of the latter, the intermediary stage that acts as the accelator to the induced reaction may be either a combination of the actor with the inductor, or a higher oxidation stage of the actor or the inductor.

The oldest known theory of intermediary reaction is that devised to explain the acceleration exerted by the oxides of nitrogen upon the oxidation of sulphur dioxide in the manufacture of sulphuric acid. The recent studies of Trautz have apparently demonstrated that there are more intermediary reactions than had been previously assumed. The sum reaction may be represented in the reaction: $2\ NO + O + H_2O + SO_2 = 2\ NO + H_2SO_4$. Trautz was able to determine experimental evidence for the following reactions:

$$NO + NO_2 + H_2O = 2\ NO.OH.$$

$$\begin{matrix} NO. \\ NO. \end{matrix} \left| \begin{matrix} OH + H \\ OH + H \end{matrix} \right| \begin{matrix} SO_3H \\ SO_3H \end{matrix} \longrightarrow (2\ NO.SO_3H + 2\ H_2.) \longrightarrow NO \begin{matrix} \diagup\ SO_3H \\ \diagdown\ SO_3H \end{matrix} + NO + 2\ H_2O.$$

$$NO \begin{matrix} \diagup \\ \diagdown \end{matrix} \left| \begin{matrix} SO_3H + OH.NO \\ \\ SO_3H + OH.NO \end{matrix} \right| \begin{matrix} NO \\ \\ NO \end{matrix} \longrightarrow 3\ NO + 2\ H_2SO_4.$$

$$2\ NO + O + 2\ H_2SO_4 - 2\ NO_2.SO_3H + H_2O.$$

$$2\ ONO.SO_3H + 2\ H_2O + NO(SO_3H)_2 - 3\ NO + 4\ H_2SO_4.$$

$$\begin{matrix} ONO \\ +\ H \end{matrix} \left| \begin{matrix} SO_3H \\ -HO \end{matrix} \right. \longrightarrow NO.OH + H_2SO_4.$$

$$2\ NO + O_2 = 2\ NO_2.$$

The catalysor NO acts to a certain extent as an anti-catalysor. The distinction from the coupled reactions is apparent. The most interesting phase for us lies in the fact that the catalysor NO reacts not only with the oxygen and the substrate SO_2, but also with the product SO_3, and that from the reactions between the product and some of the higher reaction-stages a portion of the velocity of the process is derived. This is of importance in furnishing an inorganic illustration to what is undoubtedly of frequent, possibly of regular occurrence, the reaction of combination between the ferment and the product of its acceleration.

A closely analogous condition in the organic work seems to lie in the phenomenon of the action of acids upon the formation of isomers of cinchonin as described by Skraup. When cinchonin is exposed to the action of hydrochloric acid (or other halogens), one isomeric base is produced, the *a-i*-cinchonin, and the HCl addition-product of cinchonin.

$$\text{Cinchonine} + \text{HCl} = \begin{cases} \text{HCl—cinchonine (addition reaction)} \\ a\text{—}i\text{—cinchonine (transformation reaction)} \end{cases}$$

Secondary reaction: a—i—cinchonine + HCl = HCl—a—i—cinchonine.

The first idea would naturally be that suggested by Wislescenus for the transformation in such reactions, that the addition-product represented the intermediary product. Skraup, however, has shown that the addition product with HCl is not to be converted into HCl and the isomeric base under the conditions of the experiment. The reaction has since been considered from the kinetic point of view by Wegscheider,[2] who interprets the relations as follows. When HCl and cinchonin are brought into a system two reactions occur, probably in definite proportions and in accordance with the law of mass action; the end products of the two reactions are firstly the HCl addition product of cinchonin, and secondly the isomeric base, *a-i*-cinchonin. The first reaction or product acts in some way as the catalysor for the second reaction, the transformation into the isomeric base. It is certain that the concentration of the hydrogen ions does not determine the velocity of the formation of the isomeric base. The accelerating influence of the side reaction of addition upon the reaction of transformation may be regarded as one of two procedures.

Either some intermediary form of the addition-reaction constitutes an intermediary form of the transformation-reaction; *i.e.*, in the series of intermediary forms of the addition-reaction is a point where the process may go on to the reaction of transformation, a point where the line of least chemical resistance lies in the direction of the transformation-reaction; or the two lines of direction are early separated and some product of the addition-reaction acts as a catalysor to the transformation-reaction, and produces with this reaction intermediary forms that carry with them a heightened velocity of this second reaction. Wegscheider inclines to the view that the first reaction alone, the addition-reaction, is an auto-reaction; he believes that the second reaction is not one that exists per se and is simply accelerated by the first reaction, but that the first reaction or its products actually calls the second reaction into being.

We meet here with an apparent contradiction of the statement that a fermentation is an acceleration of an already existing reaction. If in a catalysis or fermentation the end product is different than that yielded in the unaccelerated reaction, the relations suggest a reaction de novo. When the relations are carefully scrutinized, however, it seems clear that we are dealing not with a contradiction, but with an extension of the principle. van't Hoff has pointed out that in some catalytic reactions the factor of internal chemical resistance has been such as to inhibit the unaccelerated reaction; but he does not term such a catalytic reaction a reaction de novo. In a similar manner, even though in some instances the end product be different in the accelerated and the unaccelerated reaction, it is the existence of the primary unaccelerated reaction that makes possible the secondary reactions in the process of acceleration that yields the end end products. In the domain of organic substances the liability is so great and the possibilities of reactions so numerous that many possibilities for the installation of side reactions are presented in the catalyses and fermentations of such substances. This may be illustrated in the following scheme:

Substrate + water = p_a = p_b = p_c = end product A. (auto-reaction).
Substrate + water + ferment
= p_r = p_s = p_t = p_u = end product A. (accelerated reaction).
└——— = p_y = p_z = end product B. (side reaction).

Obviously the side reaction is not a reaction de novo, but is as essentially an acceleration (and deviation) of an auto-reaction as is the accelerated reaction that yields the same end product as the auto-reaction. And were the entire trend of the reaction to take the side path and product *B* appear as the sole end product, that fact would hold just as true. In many of the cases we are dealing with uncompleted or superimposed reactions. Thus sugar may apparently be fermented to alcohol and to acetic and lactic acid. Now there can be little doubt that the lactic acid fermentation consists in the reaction as described for alcoholic fermentation checked at the stage of lactic acid; and the acetic acid fermentation is an oxidation fermentation of alcohol. Up to the present therefore we have no data tending to indicate that fermentations are ever reactions de novo.

An interesting form of catalysis, and one doubtless of widespread occurrence in the inorganic world, consists in the literal formation of a galvanic cell within the reacting system, the catalysor not participating in the chemical reaction directly. A good illustration is seen in the action of copper upon the solution of metallic zinc by sulphuric acid. The reaction with the pure substances follows the following equation:

$$H_2SO_4 = 2\ H^+ + SO_4^=.$$
$$Zn + 2\ H^+ = Zn^{++} + H_2.$$
$$Zn^{++} + SO_4^= = ZnSO_4$$

This reaction is slow, as is familiar to every one who has attempted the preparation of hydrogen from the pure reagents. A trace of copper will accelerate it greatly, by the formation of a galvanic cell with a closed circuit.

Anode Zn. $Zn = Zn^{++} + 2\ \bar{U}.$
$Zn^{++} + SO_4^= = ZnSO_4.$

Cathode. Cu | $H_2SO_4 = 2\ H^+ + SO_4^=.$
$2\ H^+ = H_2 + 2\ \overset{+}{U}.$

In closed circuit: $2\ \bar{U} + 2\ \overset{+}{U} = 0.$

The reaction is very rapid, and is doubtless illustrative of frequent disintegrations of impure metals.

For organic reactions intermediary products have been less often demonstrated, on account of the greater complexity of the relations. Some are, however, known. The first demonstrated instance (which is now known to be susceptible of marked catalytic acceleration) was made by Williamson for the formation of aether from alcohol through the action of sulphuric acid, aether-sulphuric acid being shown to represent the intermediary stage.

$$H_2SO_4 + C_2H_5.OH = (O_2H_5)H\ SO_4. + H_2O.$$
$$(C_2H_5)\ H\ SO_4 + C_2H_5.OH = (C_2H_5)_2O + H_2SO_4.$$

When propyl alcohol is heated with sulphuric acid, a molecule of water is withdrawn; thereupon another molecule of water is added, though in a different way, so that isophopyl alcohol is formed.

$$CH_3.CH_2.CH_2OH - H_2O = CH_3.CH:CH_2 \text{ (propylen).}$$
$$CH_3.CH:CH_2 + H_2O = CH_3.CHOH.CH_3.$$

The formation of acroline from glycerine by heating is illustrated in the following series:

$$\left\{\begin{array}{l} CH_2.OH \\ | \\ CH\ \ OH \\ | \\ CH_2.OH \end{array}\right\} -2\ H_2O = \left\{\begin{array}{l} CH_2 \\ | \\ C \\ \| \\ CH\ .OH \end{array}\right\} + H_2O = \left\{\begin{array}{l} CH_2 \\ | \\ CH \\ | \\ CH.(OH_2) \end{array}\right\} - H_2O = \left\{\begin{array}{l} CH_2 \\ | \\ CH \\ \| \\ CH.O \end{array}\right\}$$

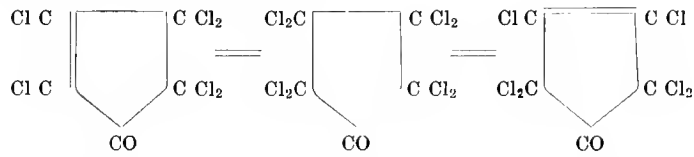
glycerine — acrolein hydrate — acrolein

According to the generally accepted theory, when water and carbon dioxide are in contact in the presence of sunlight formaldehyde is slowly formed, and the acceleration of this reaction is supposed to constitute the first step in the assimilation of carbon by plants. The reaction may be regarded as passing through the intermediary stage of formic acid.

$$H_2O + CO_2 = H.COOH + O.$$
$$H.COOH = H.COH + O.$$

An interesting catalytic intramolecular transformation described years ago by Zincke and Kuester affords another good illustration. The ketone of C_5Cl_6O presents several isomers, and two of these in particular tend always to pass into each other and to establish an equilibrium in the mass. The reaction is apparently not direct, and although the intermediary body has not

been isolated, the evidence seems to indicate that the reaction follows the following equation:



An additional illustration is furnished in the equations for the fermentation of d-glucose to alcohol and carbon dioxide, given in the lecture on alcoholic fermentation. There the one intermediary product is lactic acid, which has been confirmed, while the first is still unknown, though a glyoxylic body seems recently to have been identified in the intermediary series. These examples could be multiplied by illustrations from the literature. The sole reason why the general theory of the occurrence of intermediary stages in all reactions cannot be confirmed in each concrete instance lies in the instability of these products and in the transitoriness of their appearance.

Apart from the considerations adduced for alcoholic fermentation (and for the oxydases and peroxydases) there have been few studies of fermentations from the point of view of intermediary reactions. As a rule the conditions are so complex and uncontrollable that we do well if we are able to estimate the march of the reaction and the nature of the final products, without even attempting the isolation of intermediary reactions. In the fermentations of the hexoses, in the platinic accelerations of cleavages of carbohydrates, and in the reactions of hydrogen peroxide with organic bodies the relations promise soon to be sufficiently clear to permit of investigations from this point of view. We know that ferments are very labile bodies, that precisely such as labile bodies have the tendency to enter into combinations of high lability, and we may infer that in this very quality lies their adaptability to the acceleration of slow reactions. But this very lability makes the intermediary products so very elusive and incapable of isolation, so that qualitative results are probably all that may be hoped for in the near future. Schoenbein spoke of the reactions of hydrogen peroxide as the "Urbild

aller Gaehrung,'' and this terse sentence is yearly becoming more impressive. There was a time when the reactions of hydrogen peroxide were as little understood as are those of the common ferments to-day, and it is not too much to hope that as much progress may be made with the latter within the next decade as has been made during the last three decades upon the study of the catalyses with hydrogen peroxide. To this end, however, we shall need to prepare ferments in a much greater state of purity than is now possible, in order that secondary and extraneous reactions shall be excluded from the system.

Although we are not as yet able in concrete instances of fermentation, apart from alcoholic fermentation, to point out the intermediary reactions, we have an indirect argument for this theory in the fact that the ferment is known to combine with the substrate. While it is true in the general sense that the action of catalysors is peculiar in this, that there is no stochiometric relation between the catalysor and the ferment, it is, on the other hand, equally true that on the theory of intermediary reactions, during the moment of reaction there must be a stochiometric relation between them. The statement that there is no stoechiometric relation between the catalysor and the reaction it accelerates is true only in the relative sense that there is no stochiometric relation between the mass of the catalysor and the mass of initial substrate or the final products. But so long as we locate the modus operandi of catalytic acceleration in intermediary reactions, there must obviously be a stochiometric relation between the catalysor and the substrate in the moment of reaction. This is as true of colloidal platinum as it is of ferrous sulphate. It is the rapidity of the intermediary reactions, the putting-on and casting-off of the reaction, so to speak, that gives the gross appearance of absence of a stochiometric relation. Only on such a basis can the relation of degree of acceleration to mass of catalysor or ferment be explained. It is my conviction that when the common fermentations, the reactions of monomolecular order, are carefully studied, it will be found that in all the degree of acceleration is proportional to the mass of the ferment. This is just what we should expect, since the only relations in the reaction are the masses of the substrate and the ferment. It might be assumed

that the molecules of ferment f each combined with one of substrate s. This would give us $f + s = fs =$ intermediary product (one or more) = end product $+ f$. This f would then combine with another molecule of substrate, and the process be repeated. On the basis of this scheme, the degree of acceleration would naturally be proportional to the number of f, at least within certain limits of relations of concentration.

There have been several other considerations urged to explain the accelerations of catalysis and fermentation. These theories do not exclude the proposition of intermediary reactions. Euler has urged the application of the theory of ionization to this entire group of reactions. His hypothesis, which, though unsupported by much experimental evidence, is founded upon solid general considerations, regards the action of the catalysor as a positive influence on the magnitude of ionization of the reacting bodies. The presence of the reacting body is considered to so alter the concentration of the active ions that the reaction is hastened. "Chemical catalyses depend upon alterations in the concentrations of one or more of the molecules that carry on the unaccelerated reaction, *i.e.* (by the application of the electro-chemical principle to the general field of chemistry), upon an increase (or a decrease) of the ions that participate in the reaction."

The hypothesis of Bodenstein rests upon the proposition that there exists about the catalysor a zone of increased concentration through which the velocity of the reaction would be hastened. This hypothesis is obviously most applicable to catalysis within a heterogeneous system.

The validity of the general principles underlying these two hypotheses cannot be denied. At the same time, it cannot be believed to-day that they alone can explain a catalysis or a fermentation, independent of the existence of intermediary reactions.

The work of Bredig and his pupils on the catalytic action of colloidal suspensions of metals has tended to exaggerate the physical aspect of the subject. Granting unreservedly the accessory influences that the physical properties of colloidal suspensions (their enormous surface tension, etc.) may possess upon a reacting system, the fact remains that the catalytic influence of colloidal metals must, too, be attributed to intermediary reactions.

The colloidal state must be held to endow the metal with activity in the chemical sense, to activate it in the mass sense. A gross suggestion of such a process is contained in the proposition that if a metal were chemically active in itself, the finer the subdivision of the metal in the system, the greater the surface exposed for contact. Thus copper and platinum are slightly catalytic in sheet form, enormously active in colloidal state. Whatever the process may be, we may be sure that the catalytic property of colloidal suspensions lies in the chemical activity of the substance in that state. The physical properties of colloids, especially of the stable organic colloids, are indeed difficult of definition and characterization, and almost impossible of control; but that is no reason why the "colloidal properties" should be blindly invoked as an explanation of whatever may appear obscure. The fundamental fact in the phenomenon of fermentation is a chemical act; and howsoever the physical conditions of the reacting substances and the system may modify that reaction in one direction or another, they cannot supplant the chemical reaction as the fundamental fact of the phenomenon.

LITERATURE.

Bach and Chordat. Biochemische. Centbl., *1*, 417, 457.
Bodenstein. Zeitschr. f. physik. Chem., *46*, 725-*49*, 41, 61.
Bredig. Die Anorganische Fermente., 1901.
Brode. Zeitschr. f. physik. Chem., *37*, 257-*49*, 208.
Engler and Weisberg. Krit. Studien u. d. Vorg. d. Autoxydation, 1904.
Euler. Zeitschr. f. physik. Chem., *36*, 641-*40*, 498-*47*, 353.
Berichte, *33*, 3202.
Compare also Wegscheider. Zeitschr. f. physik. Chem., *39*, 257-*41*, 162.
Kastle and Loevenhart. Am. Chem. Jour., *29*, 397, 563.
Loew. Arch. f. d. ges. Physiol., *27*, 203.
Science, 1899, 955.
Luther. Zeitschr. f. physik. Chem., *43*, 203-*46*, 777.
Pollok. Zeitr. z. chem. Phys. u. Path., *6*, 95.
Schilow. Zeitschr. f. physik. Chem., *42*, 641.
Trautz. Zeitschr. f. physik. Chem., *47*, 513.
Wegscheider.[1] Zeitschr. f. physik. Chem., *30*, 593. Also Ibidem., *34*, 290-*35*, 565.
Wegscheider.[2] Zeitschr. f. physik. Chem., *34*, 290, *35*, 565.
Kuester. Zeitschr. f. physik. Chem., *18*, 161.

THE SPECIFICITY OF FERMENT ACTION.

In the question of the specificity of ferment action we are confronted with a problem of great importance. Like many problems, it has become appreciable but gradually, with the development of the collateral aspects of the general study of ferments. In the routine text-books on physiology one may still meet with the statement that the specific limitations of the power of ferments, the ability to ferment but one or at most nearly allied bodies, consitutes a distinction between ferments and inorganic catalysors. Nothing could be farther from the truth. There are many instances of quite specific action among inorganic catalysors. For example, iron salts act as good catalysors for the oxidation of potassium iodide by a persulphate; but they will not accelerate the reaction of the same persulphate upon sulphurous acid. Wolframic acid is an active catalysor for the oxidation of hydriodic acid by hydrogen peroxide, but it will not accelerate the same oxidation by a persulphate. And in a similar manner potassium bichromate will accelerate the oxidation of hydriodic acid by bromic acid, but not the oxidation by iodic acid. Platinum black is a good accelerator for the hydrolysis of esters of the simple alcohols, but it has no appreciable effect upon the hydrolysis of esters of glycerine. On the other hand, trypsin will digest many different proteins, and even many synthetic poly- and dipeptides. Laccase will accelerate the oxidation of many aromatic bodies, and the reduction ferments will accelerate many different reactions of reduction. A simple contemplation of the chemical relations concerned leads to the view that since these accelerations are to be regarded as founded upon intermediary reactions, whether a ferment acts or not will depend solely upon the particular reaction involved. The dissociated hydrogen ions are indeed quite general catalysors for reactions, but they do not share this generality of action with many inorganic catalysors. An interesting exception to the general rule that hydrogen ions act as general catalysors is found in the observation

that acids are not able to convert adenine and guinine into xanthin and hypoxanthin, though these are reactions of hydrolysis. All catalysors may be said to be more or less specific; the inorganic catalysors are less specific (*i.e.*, have a wider range of availability in the inauguration of intermediary reactions) than the organic ferments. The specificity itself must theoretically be vested in the chemical relations of the intermediary reactions.

In a discussion of the specificity of ferment action we must distinguish between quantitative and qualitative specificity. By quantitative specificity we mean whether a ferment does or does not accelerate a certain reaction. By qualitative specificity we mean that a ferment not only accelerates a reaction, but so modifies it as to determine the chemical nature of the products.

In the beginning it must be pointed out that the fermentability of a certain body may be only a relative term, with a time limitation. When we say that a certain ferment will not act upon a certain substance, we usually mean that a test of several hours or days is made and then the results determined with a certain analytical precision. The accuracy of the observation depends upon the purity of the reacting bodies, the stability of the ferment, the length of time permitted, and the delicacy of the analytic procedures used to determine the occurrence of the reaction. It is apparent that a ferment could act in a positive manner, but that the acceleration might not be measurable under the chosen or necessary conditions of the experiment. In a strict sense one ought to demonstrate that the velocity of the reaction with the ferment of supposed inactivity possesses the same velocity as the simple system without ferment. On the other hand, a positive result might be spurious. For example, pentoses are not fermentable with zymase; but an appreciable quantity of alcohol and carbon dioxide could appear in such an experiment, derived from the glycogen contained in the extract of the yeast. In a review of the reported work one is impressed with these facts: As a rule the time has been too short; the analytical methods for the determination of the occurrence of a reaction have been often coarse; and the reacting bodies and ferments have rarely been pure enough to insure an unequivocal interpretation of positive or negative results. If the successful reversion of ferment action

had been done in the routine manner of testing for ferment action, not a single one of the now demonstrated reversions would have been discovered.

Many ferments have but a limited range of activity; they are able to ferment but certain few substances. When we say that a ferment is able to act upon a certain body, we mean to a measurable degree. For example, trypsin is able to ferment protamine; pepsin is not able to ferment it. By this I mean that in a test of several weeks no demonstrable quantity of arginine may be recovered from the system. For many other ferments, however, the situation is different, in that a very slow fermentation occurs. Thus pepsin ferments reticulin with difficulty, trypsin with still greater difficulty, so that the current statement is that reticulin may not be fermented with trypsin. In all probability the true statement would be that outside of the fermentations of the carbohydrates, which have been best studied, all statements of non-activity applied to ferments usually mean that under the conditions of the experiment, in the short life of the ferment, no appreciable reaction occurred; and it is not equivalent to the physico-chemical statement that such and such a ferment is not a catalysor for such and such a reaction.

In the case of the sugars, however, the experimental data is much greater in amount, and of good quality. Especially have Emil Fischer[1] and his students worked with great detail and deep insight into these problems. He has collected his experiences into a general induction, which rests the fermentability of sugars upon their own sterioisomeric configuration and upon an appropriate assumed sterioisomeric configuration upon the part of the molecule of ferment. Fischer studied early the commonly known facts that certain yeasts will ferment only certain sugars, and studied these relations in a systematic manner. He realized, however, that no generalizations could be based upon such living experiments alone, and he repeated the experiments with the powdered or expressed ferments. He further realized that to make the experiments convincing, the configuration of the sugars under study must be undoubted, and to fill this requirement he employed for his crucial experiment synthesized sugars.

The fermentability of a sugar depends, according to this hypothesis, upon the sterioisomeric configuration of its own molecule and of the molecule of the ferment. This statement is in reality not revolutionary. Many facts in the chemistry of the sugars indicate that the resistance to reactions and the reaction ability is allied not solely to the structural, but also the sterioisomeric configuration. The different hexoses present widely varying relations to the different compounds of hydrazine; the different osazones and hydrozones vary widely in their solubility, velocity of formation, stability, etc. The resistance of different sugars towards simple reagents like acids display also variations. Thus maltose is most easily hydrolyzed by acid, cane sugar next, and lactose most difficultly of all. Two molecules of d-glucose unite to form a disaccharide, maltose; but two molecules of d-laevulose or of d-galactose do not unite to form disaccharides, though each of them will unite with d-glucose to form disaccharides, but do not unite with each other. From the point of view of fermentations as accelerations through intermediary reactions, the Fischer hypothesis is very feasible, since the configuration might naturally be supposed to be of marked or even dominating influence in such intermediary reactions. The specificity of the ferment lies in the coadaptation of the configuration of a particular ferment for certain sugars, just as hydrogen disulphide is a specific precipitant for certain groups of metals.

For the members of the benzol series a large number of instances are known in which reaction affinity is dependent upon or associated with a certain configuration. The location of radicals in a benzol body determines often the resistance to chemical reaction displayed by that body, in that the substitution of hydrogen is not effected with the same readiness when the radicals occupy different positions. Thus substitution by sulphur radicals is easy in meta-xylol, less ready in ortho-xylol, and difficult in para-xylol. In the case of substitution by nitric acid, on the contrary, as, for instance, in the action of nitric acid upon the isomeric nitrotoluols, the reaction is most easy in the ortho- and most difficult in the meta-nitrotoluol. The oxidation of a lateral chain to a carboxyl group is likewise related to the configuration; ortho derivatives resist the action of chromic acid entirely, while

para derivatives are quite susceptible; thus ortho-brom-benzyl-bromide is entirely refractory to chromic acid, while the para-brom-benzyl-bromide is easily oxidized. And for the same reason benzyl-chloride is more easily oxidized to benzoic acid than is toluol. Similar relations exist for the splitting off of the carboxyl group: in ortho- and para-oxybenzoic acid this may be accomplished by hydrochloric acid, which will, however, fail with the meta-oxybenzoic acid. In the case of the chlorhydrates of nitro-anilines, the dissociation varies; at ordinary temperature the ortho derivative is dissociated to 10 per cent., the para to 5 per cent., and the meta derivative to but less than 1 per cent. In an analagous manner, the catalytic action of metals in synthetic reactions with aromatic bodies illustrates a certain specificity; thus in the sulphuring of anthrachinons in the presence of the salts of mercury, sulpho acids of the *a* series are formed in large part, which is not true in the presence of other heavy metals. Another illustration is the action of boric acid in the synthesis of poly-oxy-anthrachinons. Not only is there a relation of specificity between the configuration of the reacting aromatic body and the metal, there is also a specificity in the resulting product, and in a general sense under these circumstances these metals might be spoken of as catalysors that not only accelerate the velocity of the reaction but also modify the products. Illustrations could be adduced in numbers from the chemistry of the benzene series in which the interreaction of two ring compounds is dependent upon an appropriate configuration of the two molecules.

A very striking illustration of the relations of reaction acceleration to configuration is to be noted in the recent studies in photochemistry. The results of the studies of Ciamician and Sachs and their respective students seems to point to the fact that the sensibility to light upon the part of aromatic bodies is noted only in such bodies as possess a nitro group in the ortho position to a CH_2 group. Now many of these bodies are fermentable, and the fermentative accelerations bear similar relations to the configuration. Thus laccase will accelerate the oxidation of hydrochinon (para-dioxybenzol), but will not ferment the ortho- (pyro-catechin) or the meta-dioxybenzol (resorcin). Tyrosinase,

furthermore, will ferment metatoluidine, but not the ortho- or para-toluidine, while it will ferment all three of the zylenols.

If now invertase be supposed to possess a sterioisomeric configuration simply because it bears experimentally specific relations to the fermentation of particular sterioisomeric molecules of sugar, laccase and tryosinase might be supposed to bear certain configurations corresponding to the ring structures of the aromatic bodies they ferment. Yet in the case of these two last ferments this cannot be conceded, because identical specific relations apply to chromic, nitric, and sulphuric acids, which can possess no such thing. This same general consideration will apply to large numbers of ferments, like urease, arginase, lipase, animal oxidase, the acetic acid fermentation of alcohol and aldehyde, nitrification and denitrification, the bacterial reductions of metallic oxides or salts. In all of these such a relation between configuration of substrate and ferment cannot be claimed. Fischer has himself never claimed for his theory that it would fit all classes of fermentations, though this has been almost unconsciously assumed by nearly all of the writers upon the subject, particularly by the biologists.

Other suggestive illustrations of the relations between configuration and reaction ability are to be seen in the esterification of different benzoic acids. As Victor Meyer has shown, the replacement of the hydrogen atoms in the ring results in a reduction of the tendency to the reaction, due to the absence of the hydrogen, which accelerates reaction. The measurement of the reaction is accomplished by introducing the benzoic acid into an excess of methyl alcohol saturated with hydrochloric acid. But the relations of the different hydrogens are not identical. The carboxyl group being placed in the position of 1 in the ring, if the hydrogens are replaced from 2 and 4, or from 3, 4, and 5, over 95 per cent. of ester will be formed; while if the two hydrogens adjacent to the carboxyl group are replaced, at 2 and 4, or at 2, 4, and 6, almost no ester, less than 5 per cent., will be formed. Of influence further is the mass of the radicle that replaces the hydrogen in the ortho position to the carboxyl group; thus bromine and iodine with their heavy molecular weights depress the esterification, while methyl has but slight inhibitory

effect. The influence of the substitution of the hydrogen in the ortho position has been also well shown in the studies of Goldschmidt, who showed also that when, as in phenyl acetic acid, hydrogens were attached to both the carbons that carried the carboxyl groups, the reaction velocity was high, while in benzoic acid, which has no such hydrogens, the reaction velocity was low, only about 1 per cent. of that of phenyl-acetic acid. These various relations are probably best interpreted to mean that substitution of hydrogen by radicles results in increased resistance to reaction, and that this resistance is very different for different positions in the ring. That analogous relations hold for simple compounds is to be noted in the fact that fumaric acid does not tend to the formation of the anhydride, while the isomeric maleic acid does tend to the formation of the anhydride; this van't Hoff ascribes to the fact that in maleic acid the carboxyl groups are adjacent, while in the fumaric acid they are separated. This relation of the tendency to anhydride formation to the relative situations of the carboxyl groups seems to hold in many compounds. Another illustration of the relation between configuration and reaction ability has been furnished by Fischer himself, who has shown that in compounds that are asymmetric by reason of the attachment of a glucose fraction, radicles such as HCN may be attached in an asymmetric manner to the carbonyl group.

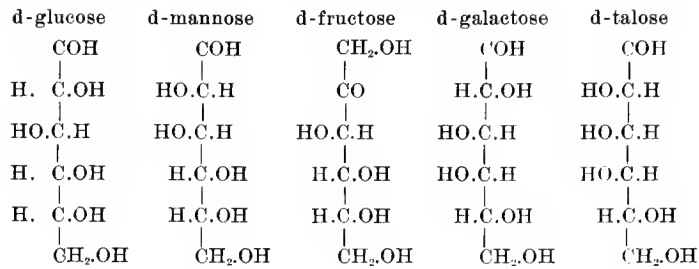
The theory of Fischer rests upon the general proposition that for the production of an optically active substance the intervention of an asymmetric optically active substance is necessary. The accepted hypothesis bearing upon the derivation of the asymmetric sugars and glucoses in nature rests their synthesis upon a photochemic process. The solar light is known to contain linear polarized light at the surface of our sphere. When this light impinges upon the surface of the seas, a certain production of circumpolarization is held to occur. The relations of the earth's magnetism are held accountable for quantitative dispersion of this circumpolarized light. The action of this light is held to account for the synthesis of asymmetric and therefore optically active carbohydrates.[1] Whether the synthesis of asymmetric sugars occurs directly in the plants under the influence of circumpolarized light, or through the agency of asymmetric substances

in the chlorofyl granules, as Fischer believes, is immaterial to the general theory, since the asymmetric chlorofyl substance would need to be derived in the same manner through the agency of circumpolarized light. Of course the converse of the proposition that only asymmetric bodies can produce an asymmetric substance—that only an asymmetric body can build down another asymmetric body—is not stated. But among the reaction possibilities of an asymmetric substance we might most reasonably suppose that some reaction tendencies as well as resistance to reaction might be vested in the sterioisomeric configuration, and would also correspond to the sterioisomeric configuration of the second reacting molecule. This relation of the two reacting molecules Fischer compared to the relations between a lock and key; and as a purely symbolic illustration he compared the lateral recessions of the lock and the lateral projections of the key to the lateral arrangements of the elements upon the carbon chain of a sugar molecule. For Fischer this arrangement meant simply a correlation favorable to interreaction in the purely chemical sense. It has been unfortunate for the future development of our knowledge of this and allied subjects that the chemical terminology was appropriated by Ehrlich and applied to phenomena of a different order and magnitude, of which the physical and chemical properties are almost entirely unknown. As Ostwald has put it, the lock and key hypothesis is being applied to all sorts of phenomena, because everybody knows what lock and key are. Indeed, Ehrlich has admitted that his use of the term lateral chain is philological and not chemical. The Ehrlich hypothesis is not a physical or chemical hypothesis at all, but simply a verbal scheme of explanation along the line of least resistance, the true verbality of which has become fully apparent only since some of the phenomena concerned have been subjected to objective physico-chemical investigation, and since Ehrlich has expanded his hypothesis to attempt to explain with it all the nutritional processes of the body.

In another direction the wording of the Fischer hypothesis and in particular its interpretation by the biological world has been unforunate. Although Fischer does not state so specifically, the general wording of his writings suggests that it is the

presence of the ferment that inaugurates the reaction of fermentation. One would assume from the descriptions given that when the isomeric methyl-glucosides are tested with the different ferments, it is the ferment that gives the occasion for the installation of the reaction. Stress is constantly laid on the sterioisomeric configuration in the positive sense, in the direction of the faculty of reaction. Now these reactions are theoretically and experimentally known to be not new, but accelerated reactions; the ferment does not act as a key to open a molecule to a new reaction. The influence of the sterioisomeric configuration lies in the opposite direction. Certain configurations endow the molecule with a great marked resistance to the reaction; the ferment modifies this internal resistance. The facts are of course the same in both instances, but the point of view is important. The reaction between a toxine and an antitoxine is of course a reaction de novo, and this constitutes a fundamental difference between it and a fermentation. Despite this, however, the Fischer hypothesis has been transferred, in the verbal sense at least, to this phenomenon; and lately the Ehrlich interpretations of the facts in the domain of toxines and antitoxines have been urged in the attempt to explain the facts in fermentations. This incongruity has arisen largely from the neglect of the principle that the ferment or catalysor deals not with the impelling force of a reaction, but solely with the internal passive resistance to a reaction. It has been this same misunderstanding that has led biologists to deny the fermentative acceleration of the reversed reaction. Nowhere in the writings of Fischer is it stated that fermentations are reactions de novo and not accelerated reactions; but the current biological interpretation of his hypothesis is as stated.

There are sixteen possible sterioisomerides of hexose. Of these twelve have been isolated from natural sources or synthesized. Of these but four are susceptible of alcoholic fermentation by zymase: d-glucose, d-mannose, d-laevulose, and d-galactose. For purposes of illustration, the sterioisomeric configurations of these will be given, together with that of d-talose, which is not fermentable.



The d-glueose, d-laevulose, and d-mannose are identical in this, that the relations of the three asymmetrie atoms of carbon that are eommon to them all are alike. The other asymmetrie atom in the d-glueose and d-mannose seems to be of no determinable influenee. In the molecule of the d-galaetose there is the difference from the other three, that in the center one of the three asymmetric atoms of carbon common to them all, the center earbon has the relations of hydrogen and hydroxyl reversed laterally. The upper asymmetrie earbon is identical in its relations with the eorresponding atom of the d-glucose, but has the reverse relation of lateral attachments possessed by the d-mannose. Nevertheless these differences are not determinating, since d-galactose is fermentable by the same yeasts, though some yeasts eannot ferment it at all, and all do so with slower velocity. It is therefore not the situations of individual hydroxyls that determine the fermentability, but the total combination. Thus d-talose, whieh is non-fermentable, resembles d-mannose in the relations of the upper asymmetrie earbon, d-galactose in the relations of the two middle asymmetric earbons, and all four in the relations of the lower asymmetrie earbon. A direct rule is obviously not eontained in these faets, but somewhere in the series of asymmetrie earbon atoms of d-talose is a high passive resistance not eneountered in the others, while somewhere in the series of asymmetrie carbons in d-galaetose is a eertain degree of passive resistanee not met with in the other three. There ean be no doubt that the faets suggest a quantitative rather than a qualitative differenee. In the original hypothesis it was postulated that the sugars with earbons in multiple of three should be fermentable by the same yeasts. In this point, however, the hypothesis failed

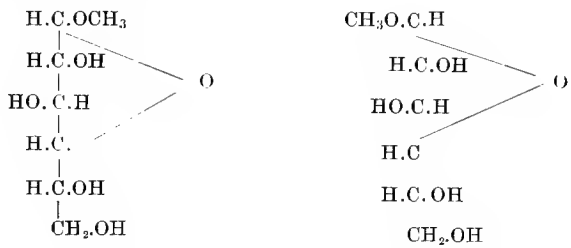
outright. The nonoses do not ferment, while the apparent fermentation of the aldo-glycerose and the keto-glycerose were shown to have been due to condensations into hexoses. (Piloty, Wohl, Emmerling.[1]) That the pentoses and other sugars do not undergo alcoholic fermentation, as usually stated, is not so certain. That pentoses are very resistant to alcoholic fermentation is true. Nevertheless it is known that certain bacteria do produce in arabinose fermentations that are accompanied by the formation of alcohol. These fermentations seem always mixed, so far as the products are concerned; lactic, acetic, formic, and butyric acids in particular are also present. (Frankland and MacGregor, Salkowski, Tollens and Schoene, Harden.) One must assume, if one clings to the assumption that the pentose is resistant to alcoholic fermentation, that the alcohol under these circumstances is derived secondarily from the real products of the fermentation. More proper chemically would it be to assume that pentose is susceptible of alcoholic fermentation by certain yeasts, but the side reactions complicate the products; thus the lactic acid could be the result of an incomplete reaction, and from it the butyric acid could be derived, while the acetic acid would represent the oxidation product of alcohol.

So far as the alcoholic fermentation of sugars is concerned we may therefore say that of the three propositions—that all sugars with carbon atoms in multiples of three, and only these are fermentable, that of the hexoses only the d-members of the sterioisomeric series are fermentable, and of these only those possessing a certain configuration are fermentable—the first is not correct, the second is correct so far as known, and the third is correct in the quantitative sense, though unproved in the qualitative sense.

Cremer has advanced a suggestion that locates the fermentability of the hexoses in their being convertible into d-glucose. He suggests that only d-glucose is fermentable, and those sugars only subject to fermentation that are convertible into d-glucose. That these hexoses may be easily converted into each other through the action of alkali has been shown by Lobry du Bruyn and van Erenstein. The formation of d-laevulose from d-glucose is according to recent investigations of Ost not confined to the experimental

conditions described by these authors, but occurs in every hydrolysis of starch, arising not from the starch directly but by polymerization of the d-glucose. The theory of Cremer has no direct or experimental proof in its favor, and is opposed by many demonstrated facts.

In the attempt to secure clearer relations, Fischer[2] tested the fermentation of natural and synthetic glucosides. When aldohexoses are heated in alcoholic hydrochloric acid, glucosides are formed. Of both the d- and l-sugars two isomers are formed, termed the *a* and *b* series. Thus for methyl alcohol we have the two isomers of d-glucose:



Fischer found that the glucosides of the l-series of glucose, mannose, and galactose were not fermentable at all. The glucosides of the d-series of glucose, mannose, and galactose were fermentable, but to a noteworthy degree specifically. The *a*-glucosides of these d-hexoses were fermentable with yeasts, the *b*-glucosides were fermentable with emulsine. The natural glucosides are *b*-glucosides, so that the concordance between the natural and the synthetic glucosides is complete. Amygdaline, however, is split by the alcoholic yeast to the extent that one molecule of d-glucose is split off; the remaining mandelnitril-glucoside, however, is fermentable only by emulsine. Whether the emulsine is able to accomplish the complete fermentation of the amygdaline, or whether a maltase is always present, is not determined. It is not the zymase in the yeast that accelerates these reactions; it is the maltase or allied ferment. The data bearing on these synthetic glucosides is very positive, and suggests strongly the validity of the Fischer hypothesis. The time allowed the tests has, however, been usually short, often only twenty hours, so that a

slight acceleration of the cleavage of the *b*-glucosides by the maltase and the *a*-glucosides by the emulsine was not positively excluded.

Similar considerations hold for the disaccharides. Invertase ferments cane sugar, and is depressed only by the d-laevulose, not by the d-glucose. Maltose ferments only maltose, and is depressed of course by the d-glucose. Neither of these ferments can split a typical *b*-glucoside or milk sugar. Lactase ferments milk sugar, and also *b*-glucosides, but no *a*-glucosides; it is depressed only by the d-galactose. Milk sugar for its part is fermentable by emulsine, which is able to ferment neither cane sugar nor maltose. This seems strange, since maltose and lactose are structurally so closely related, and have apparently closely corresponding sterioisomeric configurations. Pottevin has recently repeated the experiments, and could not find that milk sugar was fermented by emulsine or that lactase was able to ferment *b*-glucosides. This would accord much better with the Fischer theory than the earlier results of Fischer himself. As a matter of fact, these and all the other experiments ought to be repeated with isolated purified ferments, under careful conditions of experimentation.

Confirmatory evidence has been very recently obtained by Fischer[3] through the study of the tryptic digestion of synthetic peptides. Of the many synthetic peptides that have been prepared and tested by Fischer and his pupils, the following racemic substances are digestible with trypsin: Alanyl-glycin, alanyl-alanine, alanyl-leucine A, leucyl-isoserine, alanyl-glycyl-glycin, leucyl-glycyl-glycin, glycyl-leucyl-alanine, and alanyl-leucyl-alanine. When now these racemic peptides are digested with trypsine, the cleavage is an asymmetric one, and the amido acid that is split off is the same active amido acid that is to be found among the products of the digestion of natural protein by trypsine. There are two racemic alanyl-leucine peptides, and they include the four possible combinations of the two components. Thus alanyl-leucine *A* is d-alanyl-l-leucine + l-alanyl-d-leucine; while *B* is d-alanyl-d-leucine + l-alanyl-l-leucine. Only *A* is digestible, and of the compound only the d-alanyl-l-leucine is split off, and the two components separated. These facts have all the more

weight, because they have been obtained from a class of compounds totally different from the sugars.

According to the theory of Fischer, since the velocity of reaction under the influence of a ferment depends upon the steriosomeric configuration of the ferment molecule, the influence of organic acids upon the same reactions should depend upon similar relations. Fischer[4] himself tested this theory. He studied the acid hydrolysis of cane sugar under the influence of d- and l-camphoric acid; the result was negative to his theory, for the acceleration was identical in the two, *i.e.*, the acceleration depended upon the electrolytic dissociation, and not upon the optical isomerism.

Another interesting point from which to view the theory lies in the fermentation of racemic acids. Pfeffer, Purdie, and more recently Mackenzie and Harden have gone over this ground quite thoroughly. Their results are in the main in accord, in that both the d- and l-acids are fermented, but with different velocities. In some few instances the reactions upon the two enantiomorphic bodies were quite equal, in other instances there was a distinct predominance; in a few instances the reaction with the one was marked, with the other very slight. The greater velocities were in the direction demanded by the Fischer theory. If these results may be applied to the fermentation of sugars, it indicates that the specificity is only one of degree, that it is not a question of reaction or no reaction, but of slight reaction as against pronounced reaction.

Condelli has shown that the temperature optimum is different for the d- and l-acids. He suggested further that a particular yeast were able to act upon the l-acid only by first converting it into the d-acid. This is begging the question.

Dakin has studied the cleavage of optically inactive racemic synthetic esters by lipase. The products were found to be rotary in one direction, and the residual ester was rotary in the opposite direction. In a word, the cleavage was an asymmetrical process. In the various esters studied, those optical isomerides most rapidly attacked possessed similar configurations, thus conforming to the Fischer theory. But it was simply a difference of velocity, not a qualitative differentiation. The corresponding

fact to this is to be found in the observation of Markwald and Mackenzie that two optically opposite active acids do not form ester with an optically active alcohol with the same velocity. The unequal velocities are explained by Dakin as follows: When optical isomerides combine separately with the same structurally asymmetric substance, they may do so with unequal velocities; and conversely, the products formed by such reactions, since they are no longer optical opposites, might be expected to undergo further changes at unequal velocities. If the enzyme be supposed to be dextro-rotary and the components of the racemic acid be represented by $+S$ and $-S$, the additive compounds formed by the union of the ester and the ferment would be $(+ e + S)$ and $(+ e - S)$. These two compounds are obviously not enantiomorphic (opposite compounds would be respectively $(- e - S)$ and $(- e + S)$, and might therefore be expected to be formed and undergo changes at different velocities. This argument may be pursued further. Ferments are apparently sometimes active, sometimes racemic substances. If a racemic ferment react with a racemic substrate, we would expect the formation of enantiomorphic complexes of ferment-substrate, and under these circumstances we should expect the reaction relations to be identical. Under these circumstances we should expect an active acid to act in a different manner from a racemic or an inactive acid. Fischer tested this hypothesis, by the use of active camphoric acid in the inversion of sugars, with negative results, as previously stated.

There is, on the contrary, considerable data bearing against the Fischer hypothesis. For the bacterial fermentations, and especially for the lactic acid fermentations, results have been obtained that are not in harmony with the theory. Emmerling,[2] a pupil of Fischer, in his brochure on the fermentation of carbohydrates, repudiates squarely the position that a certain germ in pure culture will under all circumstances with a known sugar as substrate produce the same reaction. Upon this aspect of the question, however, little weight is to be laid. The theory of Fischer cannot be proved or disproved by cultural investigations; isolated purified ferments can alone furnish data of the quality necessary in deciding a fundamental problem. A theory of fer-

mentation it is not and in the nature of things eannot be. When it is shown that for a particular fermentation the intermediary reaetions that constitute the acceleration are dependent upon the possession of a certain sterioisomeric or other configuration upon the part of the substrate or the substrate and ferment, that constitutes a fact for that fermentation. The investigations of the future must determine how closely the facts eonform to the theory in a particular group of fermentations, and to how many elasses of fermentations the principle is applicable. It will quite eertainly be found that the relation is a quantitative one, not a qualitative one. A certain configuration is favorable to the reaction, another one much less favorable. Just as the ferments are tested on a partieular sugar, so acids may be tested. Mineral acids would give results within a few moments. Some weak organie aeids would not give measurable results for days. But the actions of the different aeids are identieal in quality, though varying greatly in quantity. Similar relations may theoretically be expeeted from the ferments.

The reason a ferment is elective in the sense of Fiseher must be aseribed to the fact that configuration means resistance to the reaction or that it means resistanee to the catalysor. van't Hoff has classified the inhibitory properties in substances that operate against the reaction velocity. These find unquestionably applieation to the present problem. They are:

(*a*) Nature of the inhibitory influences in changes in physieal state. (1) The necessity of moleeular orientation. (2) Necessity of spaeial transition. (3) Capillary influences.

(*b*) Nature of inhibitory influenees in chemical transformations. (1) The necessity of moleeular orientation. (2) The neeessity of special transition. (3) Capillary influences. (4) Inhibitory influences of undefined nature that are operative for eertain reactions and eharacteristics of them, independent of the previously enumerated factors. Of these two illustrations may be given. Fumaric acid under certain eonditions remains stable and does not pass into maleic aeid, though it is easy to show that the latter is the more stable form, and easily produced from the former by appropriate eontaet action. Here, in short, an autoreaction does not oeeur with any velocity. Secondly, we know

that there are for many substances and complexes conditions, especially known for temperature and pressure, under which the substances remain in a state of apparent equilibrium, which may, however, be demonstrably not the true equilibrium. Pélabon has shown that the system $SeH_2 = Se + H_2$ forms above 325° a true state of equilibrium; it is immaterial from which direction the reaction proceeds, the final result is the same. But below 325° this is not the case; a false equilibrium is attained, which varies for different temperatures.

These considerations apply of course not only to the problem of the specificity of ferment action, but to the general proposition that ferments are accelerations of existing reactions, and not reactions de novo.

The question of qualitative specificity of ferments has a narrower interest, though it is one of great importance. Do the different accelerators of a reaction yield the same products, or may they yield different products? It is obvious that with a pure catalysor, a body that simply diminishes the chemical resistance of a substance, the nature of the products would not be altered. But in the frequent atypical fermentations, where the ferment is often more or less altered in the course of the reaction, some alteration in the products might be expected. Such alteration might, however, not be properly attributed to the catalytic acceleration. For example, a certain reaction constitutes a hydrolysis, with the product *z*. The ferment enters into reactions with *z*, and as a result *y* is produced. Here we have a new reaction, one not connected with the acceleration, but one that would have occurred were we to mix *z* and the ferment. The reaction constitutes a new reaction, a secondary reaction in the system. An illustration of this is seen in the transformation previously described of cinchonin into the isomeric base, *a-i*-cinchonin under the action of hydrochloric acid. The product slowly adds hydrochloric acid; this is a secondary reaction having no relation to the accelerated transformation of the cinchonin into the isomer, but simply a reaction between the *a-i*-cinchonin and HCl such as would occur were these two mixed in a solution. A further possibility lies in the fact that the products may react among themselves to form new bodies, which are of course not to

be classed as products of the fermentation. For example, arginase accelerates the hydrolysis of arginine to urea and ornithin. The fermentation proceeds well at a slightly alkaline reaction. Now the alkali accelerates the hydrolysis of urea to carbon dioxide and ammonia. The appearance of ammonia in the fermentation of arginine by arginase is therefore not a product of this fermentation. We must thus bear in mind the possibilities of secondary reactions—reactions between product and ferment, between product and solvent, between products and some extraneous substance, and between products and products. An excellent illustration of the relations to an extraneous body is furnished by the Duclaux experiments on the chemical fermentation of d-glucose. When the sugar is exposed to sunlight in the presence of a trace of sodium hydroxide, aethyl alcohol was produced; when calcium hydroxide was employed lactic acid was produced. Now since the accelerator was the hydroxyl ion, which was the same in each system, the difference in product must have been due to some action of the calcium as against the sodium. It is clear therefore that only under conditions of experimentation with pure substances can we determine whether different ferments produce different products. Practically all the studies in connection with animal ferments may be regarded as worthless from this point of view. For example, the members of the Hoffmeister school believe that the ferment connected with the autolytic digestion of the liver is not trypsin, because ammonia is formed in the auto-digestion, while it is not found in ordinary tryptic digestions. Now the ways by which ammonia might be produced are so manifold that a distinction between trypsin and the intracellular ferment based solely upon that finding collapses of its own instability. The glycerine and succinic acid that occur in alcoholic fermentation by yeast were long supposed to represent integral products of the fermentation; we now know that they do not occur in the fermentation of sugar by zymase, and that in natural fermentations they are in all probability not derived from the sugar at all. For most ferments this question cannot be discussed at all, on account of lack of data.

Now theoretically a ferment may be conceded the power of modifying the reaction that it accelerates. Wegscheider in his

studies on catalysis distinctly reserved such a group of catalyses, the reactions with side influences as he expresses it, as contrasted with the ordinary accelerations, reactions with only direct influences. There has been very little experimental work done upon this aspect of the subject, but we must concede the theory of Wegscheider. A further possibility for qualitative variations lies in the fact that it may be possible for a reaction to follow more than one equation, and these may be accelerated differently by different ferments. Now in the accurate recent studies of fermentations, with the use of purer reagents and stricter conditions, it is seen that the distinctions in products tend to disappear. Pepsin we now know yields the same products as trypsin; it may not be able to digest all the same proteins, but when both digest a certain protein, the same products are formed. All the tendency of the recent study in the fermentation of carbohydrates has been in the direction of simplifying the products, and we may confidently expect that as investigations proceed many of the deviations in products commonly held to be specific to the ferment will disappear.

On the contrary, there are undoubted instances in which ferments modify the course of the reaction in the qualitative sense. The illustrations that at once come to mind are the different results that may be secured with fermentations with pure cultures of microörganisms under different conditions. Emmerling[2] has only recently emphasized the statement, based upon experimental work, that employing a constant substrate, pure cultures of a germ may not yield the same products under different external conditions of experimentation. There can be no doubt of this. But these instances cannot be directly quoted in a chemical discussion, because of the complexity of the relations. We have definite chemical illustrations. The hydrolysis of hydrolylamine in alkaline solution follows the equation: $3\ NH_3O = NH_3 + N_2 + 3\ H_2O$, while the acceleration of the reaction with platinum follows the equation: $4\ NH_3O = 2\ NH_3 + N_2O + 3\ H_2O$. The reactions of hydrazine illustrate the modifications that may be effected by other relations in the system. Thus in simply watery solution the reaction with platinum runs $2\ N_2H_4 = 2\ NH_3 + N_2 + H_2$, while in the presence of an alkali the reaction is: $3\ N_2H_4$

$= 2\ NH_3 + 2\ N_2 + 3\ H_2$. The best illustrations, however, have been recorded in the reversions, and these are of great value because the action is very selective. When lactase is allowed to act upon equal parts of d-glucose and d-galactose a disaccharide is formed; this is not lactose, as would be expected since the synthesis is the ferment-reversion of lactose cleavage; the sugar is the isomer isolactose. The same fact holds true for the synthesis by ferment action of disaccharide from d-glucose by maltase; the sugar is not maltose, but the isomer isomaltose. These modifications cannot be explained upon any other basis than that of the qualitative alteration of the acceleration by the ferment.

This question then resolves itself into one of fact. If we were to find two ferments that give in general the same products of cleavage, but which we could show yielded in their reversions different substances, we should be warranted in classifying them as distinct. Since the general trend of fermentation and catalyses tends to identical products, we should not reason from products to ferments except upon the basis of most definite experimental evidence. Upon the other hand, we must recognize that in the reactions of complex organic bodies the differences in chemical potential between the original body and the products are often slight, and thus several possible reactions may proceed side by side; thus a certain ferment might accelerate the reaction according to one equation more than according to another equation, and a different ferment might exhibit other relations. Therefore the matter resolves itself into one of fact solely.

LITERATURE.

Ciamician and Silber. Berichte, *34*, 1230, 2040-*35*, 1992, 3593, 4128-*36*, 1575-
Condelli. Gas. Chim. Ital., 34.
Cremer. Zeitschr. f. Biol., *32*, 49.
Dakin. Jour. Physiol., *30*, 253; *32*, 199.
Emmerling.[1] Berichte, *32*, 544.
Emmerling.[2] Die Zersetzung stickestoffreier organ. Substancen d. Bakterien, *25*ff.
Fischer.[1] Berichte, *27*, 2036, 2985.
Zeitschr. f. physiol. Chem., *26*, 61.
Berichte, *28*, 1433.
Fischer.[2] Zeitschr. f. physiol. Chem., *26*, 64.
Fischer.[3] Berichte, *35*, 3144; *37*, 3103.
Zeitschr. f. physiol. Chem., *46*, 52.
Fischer.[4] Zeitschr. f. physiol. Chem., *26*, 83.
Frankland and MacGreggor. Chem. News, *69*, 33.
Jour. Chem. Soc., *63*, 1028.
Harden. Chem. Zeit., *25*, 353.
van't Hoff. Vorles. u. theoret. u. physik. Chem., *1*, 202.
Lobry de Bruyn and van Erenstein. Berichte, *28*, 3078, 3085.
Rev. Trav. chim. Pays-Bas., *14*, 116, 203-*16*, 262.
McKenzie and Harden. Jour. Chem. Soc., *83*, 1514.
Markwald and McKenzie. Berichte, *32*, 2130; *34*, 469.
Proc. Chem. Soc., *20*, 41.
Ost. Zeitschr. f. angen. Chem., *18*, 1170.
Pélabon. C. r. Acad. Sc., *124*, 360.
Piloty. Berichte, *30*, 3161.
Pottevin. Ann. Pasteur., *17*, 31.
38, 1176, 3813.
Sachs. Berichte, *37*, 3425.
Salkowski. Zeitschr. f. physiol. Chem., *30*, 478.
Tollens and Schoene. Chem. Cenbl., *69*, II, 967, 1012.
Wegscheider. Zeitschr. f. physik. Chem., *30*, 593-*34*, 290-*35*, 565.
Wohl. Berichte, *31*, 1796.

THE RÔLE OF FERMENTATION IN METABOLISM.

THE PROTEIN METABOLISM.

The digestion of protein is an act of fermentation in all the essential details. Under physiological circumstances the protein is not entirely converted into amido acids; there is evidence that a certain absorption of albumose and peptone occurs. That the nitrogen needs of the body may be maintained upon a diet of amido acids, the completed products of tryptic digestion, has been clearly shown. The act of absorption is apparently an act of simple diffusion, though this is not clear for the albumoses. The conversion of the products of digestion into the proteins of the circulating fluids is not yet generally regarded as due to ferment-action. In favor of the theory that the process of reconversion is the reversed action of the proteolytic ferments is the general theory of the catalytic acceleration of reversed reactions, and the concrete demonstration of many instances of such reversibility. No other explanation has any experimental basis. Synthesis by cell action has no concrete meaning, and is in any event not incompatible with the idea of ferment reversion. That the reversions are accomplished with such great rapidity has been admittedly without adequate present explanation in the concrete sense. There are, however, two considerations that may be adduced. In the first place, there may be conditions resembling zymo-exciters. Secondly, it must be recalled that the conditions in the act of absorption are very favorable to a rapid synthesis. In the act of absorption we have the products of digestion diffusing through a colloidal membrane, a semi-pernable membrane, where the fluids lie in closely approximated very thin layers; in short, what may be conceived as representing an ideal heterogeneous system, and as underlying the theory of Nernst, the reactions under these conditions may be reasonably assumed to occur with great rapidity. The cells of the intestinal mucosa contain proteolytic ferments of great activity; we have no reason to believe that the cells purge themselves of ferment entirely; on

the contrary, it would be natural to believe that the colloidal meshes of the cells and membrane contain ferment. Now the synthesis is known to be complete in the submucus circulation; the membrane is very thin; the fluids of circulation perform a constant lavage. Under these circumstances the colloidal membrane in which the reactions are occurring is very thin, and thus the factor of the rate of diffusion is reduced to the minimum, and this, with the postulated great rapidity of reaction in the heterogeneous system, might adequately explain the apparently disproportionately rapid rate of synthesis of protein from the products of digestion. The reconstruction of the protein molecule from the absorbed products of digestion is usually supposed to occur in the intestinal mucosa. The portal blood is known to contain more amido nitrogen than the blood of the general circulation, and it is possible that some of the reconversion is done in the liver. This is confirmed to some extent, in an indirect way, by the fact of the toxicity of the portal blood when introduced into the general circulation without passage through the liver, as is seen in the Eck fistula. When one considers the lability of proteins, it is apparent that during the course of a digestion or of the subsequent synthesis abnormal side reactions might occur, and these might conceivably be concerned in dyspepsia.

The reconstruction of the protein in the body is apparently not simply the reformation of the molecule of original protein. The biological specificity of some of our body proteins indicates that qualitative alterations are effected in addition. Whether this biological specificity is bestowed upon the molecule of protein during the reconstruction from the products of digestion or afterwards in the body is not known. The fact holds for serum globulin rather than for serum albumin.

It is not yet known just what products of the digestion of protein are absorbed. It is certain that products are formed in the stomach that do not give the biuret reaction. In the intestine many amido acids are known to occur. There is, however, a fraction of the protein molecule that seems to resist the digestion with pepsin and trypsine, a polypeptide-like substance, that may be shown on acid hydrolysis to contain amido acids. Whether erepsine can split this is not known. Whether this could be absorbed

is not known. On the other hand, there is evidence that the lower albumoses and peptone may be absorbed. There is no demonstration yet that in natural digestion the molecule is split entirely to the simplest amido acids. It is not at all necessary, whatever may be the theory of the origin of the reconstructed body protein, to assume that the digestion of protein must carry the disintegration to the very simplest amido acids. It is easily possible that group nuclei may be condensible to protein. An illustration may be found in nuclein. Here the group nuclei are a pentose, a pyrimidine body, purin bases, and phosphoric acid. There is every evidence that the body can form nuclein from these group radicals; there is no evidence that the body can form nuclein from the decomposition products of these groups. Yet this is what is practically postulated in the assumption that protein may be formed only from the final simple amido acids, and not from groups of amido acids. The process of reconstruction may be reasonably divided into two steps: the recombination under the influence of the ferment, and the reconstruction of the molecule with the endowment of the biological properties of the particular species. Whether this be done in one or two stages is not known. For some proteins (serum albumin) it seems certain that there are no biological specificities, just as there are none for the body sugar and fat. It might be conceived that the simple condensation of the products of protein digestion occurred in the intestinal mucosa under the influence of the proteolytic ferment; and that the biological reconstruction occurred later in the liver and other body cells. The latter might, on the other hand, be as easily conceived to occur in the intestinal mucosa, either under the influence of the reversing ferment (a side reaction), or of other constituents of the cell. Whether trypsine or erepsine is concerned in the reversion is not known. That the reversed action of a ferment may lead to a product qualitatively different from the original substance has been shown for maltase and lactase. Maltase accelerates the cleavage of maltose into d-glucose; when it accelerates the reversed reaction, the product is not maltose, but isomaltose. The corresponding fact holds for lactase. This furnishes an experimental analogy for the supposition that the biological stamp is placed upon a body protein during the act of

synthesis in the intestinal mucosa. It is a current misconception that the theory of the reconstruction of the protein by the ferment action must imply that a different chemical reaction would occur if the "building stones" (the amido acids) were condensed to the protein by the action of the body cells. The essential reaction in either case is the same, and is the expression of the chemical properties of the amido acids. All that the ferment need do is to accelerate the reaction, by lowering the internal resistance to the reaction resident in the components. It might, however, in addition introduce a side reaction.

Whatever the particular proteins in the diet—casein, albumin, globuline, vegetable protein, gelatine, etc.—the protein formed from them as the result of digestion is apparently the blood protein alone; that is, serum albumin and the serum globulins. To this statement qualification must, however, be made that the ingested proteins must contain sufficient amounts of sulphur and phenyl groups in the form of compound amido acids. Thus gelatine cannot cover the full protein need of the body because it is too poor in phenyl groups; but combined with other proteins, it can cover half of the protein needs of the body.

The different proteins seem to contain nearly all the same amido acids. The quantitative relations are, however, very different with the different proteins. Under the circumstances, the differences in proteins (and in these we must include the biological properties) are in all probability to be regarded as the expressions of different intramolecular arrangements of the varying amounts of the several amido acids. Direct analogies for this view are to be noted in the different synthetic peptides, and it is indeed what should have been naturally expected from our knowledge of the sugars and the compound benzol substances. That this state of affairs tends to favor the conception of the asymmetry of hydrogen cannot be denied. The investigations of the Fischer school on synthetic peptides are of the most fundamental importance for the physiology as well as the chemistry of protein.

In general terms therefore we may speak of the conversion of these several proteins into the blood proteins as polymerizations, in the sense that the amido groupings within the molecule are rearranged. In the functions of the body now, the reverse occurs;

from the simple blood proteins are formed the different proteins of the body—casein, myogen, gelatine, reticuline, etc. Here we have the specialization of particular proteins, again by the process of polymerization. Now polymerizations under such circumstances are usually autoreactions, and they are very liable to catalytic accelerations. Analagous relations hold for the different hexoses, as will be pointed out. Theoretically it is justified to assume that these reactions of protein polymerization within the body represent fermentations.

In experiments in vitro, we learn that pepsin and trypsin are both able to accomplish the acceleration of the hydrolysis of protein to such an extent that within a number of days the substrate is in large part converted into amido acids. Complete the reaction never is. Now in the stomach the extent of peptic digestion is limited by the very short duration of the food in the stomach, so that the actual power of the pepsin does not become apparent. Under conditions of normal digestion little amido acid is formed in the stomach; indeed, after a heavy meal all of the protein will not be converted into non-coagulable protein, and at no time is the production of albumose and peptone very noteworthy. This is simply a matter of time, for if the ends of the stomach be ligated and the contents retained for a number of hours, it will be found that an extensive digestion has been accomplished. Thus we learn that, although the secretory activity of the stomach could perform a completed digestion if accorded sufficient time, in fact the chief function of the stomach (apart from a possible peptic preparation for tryptic and ereptic digestion) seems to be to mix the food and discharge it gradually into the small intestine. This knowledge of physiology is entirely in accord with the general experience in the treatment of gastric disease, that the motility of the stomach is its most important function. The power to secrete normally is an invaluable sign of the integrity of the organ, but the loss of the secretory power with the conservation of the motor power may be of little injury to the individual, while the loss of the motor function usually leads to disturbances of grave consequences. The actual hydrolysis of protein is in practice accomplished largely by the trypsin and by the erepsin, and this hydrolysis is far in excess of anything

we can accomplish in experiments in glass. The normal digestion is able to accomplish within a few hours more than can be accomplished in days with all the pancreatic juice secreted in a similar space of time. Bidder and Schmidt have shown that a cat can digest and assimilate an amount of protein one-fifth the body weight per day; but with one day's pancreatic secretion of a cat we could not in a flask begin to digest so much. This is the same experience we meet with every fermentation; we lack in the laboratory experiment some condition or substance, some zymo-exciter, that multiplies many times the velocity of the simple digestion by the secretion of the body. Something more than enterokinase or tissue substance is required to bring the velocity of a tryptic digestion in glass to the level reached in intestinal digestion. We know that the addition of succus entericus to an active pancreatic juice increases its activity, and this we must ascribe to the presence of a zymo-exciter. The regular removal of the products of digestion from the tract by absorption would of course result in the acceleration of the reaction velocity, but this factor could not account for the observed differences in velocities in artificial and natural digestions.

The catabolism of protein in the body may be with certainty regarded as fermentative. For this statement we possess three groups of evidence, and these complete the chain. We know in the first place that the end-products of the hydrolysis of protein are various amido acids. In the second place, it is possible to isolate intercellular proteolytic ferments. Thirdly, we know that the formation of urea may be traced directly to the amido acids. That amido acids are the end products of the protein hydrolysis is not only known for the test tube experiment and then applied to the cellular functions; it is also known as an experimental fact that in the bacterial and autolytic degenerations of organs amido acids are formed, and that under conditions of severe degeneration these bodies may be recovered from the tissues, blood, and urine. That urea is formed from amido acids is shown by the following facts: Many amido acids when ingested are eliminated as urea; urea may indeed be formed in the test tube by the oxidation of amido acids in the presence of ammonia; when the poly-amido acid arginine is hydrolyzed, urea is formed. It is

further known that ammonium carbonate when ingested or injected into the portal circulation is elimated as urea, and that when in dogs the liver is switched out of the portal circulation, ammonia appears in the urine in large quantities. The urine contains traces of amido nitrogen, and yields amido acids on digestion with acids. This represents apparently a fraction of the products of protein hydrolysis that has escaped through the kidneys.

The reactions whereby urea may be formed from amido acids are several. Some of these are oxidations, particularly oxidative syntheses. Of these an illustration may be given in the formation of urea from oxaminic acid. Hoffmeister, who has studied this matter most, believes that the adaptability of a compound to oxidation to urea depends upon the presence of the groups $CHNH_2.COOH$. That some of the reactions are hydrolyses is illustrated by the formation of urea from arginine. The formation of urea from ammonia, which is the reversal of the common fermentation of urea into ammonia and carbon dioxide, may be regarded simply as the result of the withdrawal of one molecule of water from the ammonium carbonate. Dreschel believed that in this process, as well as in the formation of urea from the amido acids, the reaction passed through an intermediary stage of ammonium carbamate, so that under this interpretation the formation of urea would rest upon alternate oxidation and reduction. For all of these theories there is experimental evidence, but we do not know whether one or all actually occur in the formation of urea within the body.

The formation of the urea from the amido acids and ammonia being granted as a chemical procedure, how do we know that the reaction in life is fermentative. The experimental work indicates that at ordinary temperatures and concentrations the formation of urea from amido acids and ammonia would be very slow, while in life it is rapid and capable of being greatly increased by an increase in the protein of the diet. The fresh extract of liver, as first described by Richet, will form urea from amido acids. The fresh extract of the liver, as shown by Kossel, will form urea from arginine. Confirmatory evidence may be found in the fact that several of the synthetic polypeptides, condensations of amido acids, may be hydrolyzed by trypsin. I be-

lieve these considerations warrant the statement that the formation of urea in the animal body represents a fermentation.

The known facts for the metabolic disintegration of protein may be summarized as follows. The protein is hydrolyzed to amido acids by the intracellular tryptic ferment. The amido acids may be then split, since a disamidation ferment is known to exist. The resulting ammonia is then available for the formation of urea. That urea is not formed directly from amido acids by oxidation is not denied; that it is formed by the hydrolysis of poly-amido acids, like arginine, is quite certain. Thus we have:

Protein + water = amido acids.
Amido acid + water = fatty acid + ammonia.

Ammonia –> ammonium carbamate –> ammonium carbonate –> urea. It is possible that the urea and ammonia represent a reversible system in equilibrium in the body; liver extract forms urea from ammonia or ammonia from urea with equal facility.

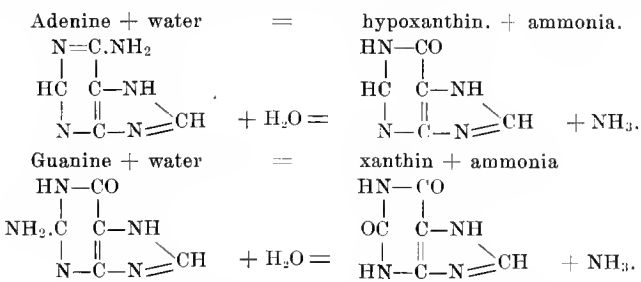
Creatinine is the anhydride of creatine, a constant constituent of muscle. It is in all regards to be looked upon as a product of the metabolism of muscle, and bears a near relationship to urea, into which it may be converted by heating with alkalies. The urinary creatinine, however, seems quite independent of the urea metabolism. It is hydrolyzed back to creatine by bacteria. The relations have been very little studied for these reactions, but there can be little doubt that what has been said of the urea applies by analogy to the creatinine, and that they are fermentative.

THE PURIN METABOLISM.

The purin metabolism is independent of the purin input in the diet. Ingested purins are hydrolyzed and the several group constituents are absorbed. Whether the absorbed purin bases are utilized in the purin synthesis is not known, but in any event the purin syntheses of the body are entirely independent of any purin input. The synthesis of nuclein comprises the combination of purin bases, pyrimidin bodies, pentose sugar, and phosphoric acid. The pentose is in all probability derived from hexose, by being built down through the removal of one atom of carbon. It is not difficult technically to build a sugar down; this

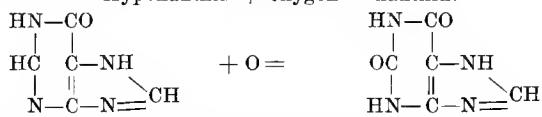
is accomplished in the method of Wohl, by converting the hexose into the oxime, which is transformed into the corresponding nitrile, from which one atom of carbon is then split off as a cyanogen group by means of silver, leaving the sugar with but five atoms of carbon. The purin bases may be synthesized from amido acids, or from urea through the mediation of tartronic acid. The pyrimidin derivatives may be formed from protein, since the pyrimidin ring can be formed from either the amido or the guanidine group. Whether these syntheses are fermentative is not known. The great importance of these syntheses has been recently emphasized for general biology by Loeb, who has indicated that fundamental problems of growth and development are directly related to the synthesis of nuclein.

Under conditions of sterilization, following destruction of the tissue ferments, nuclein is slowly hydrolyzed. This hydrolysis may be much accelerated by increase in temperature, by acids, and by bacteria. The purin catabolism is to be regarded as the enzymic acceleration of this hydrolysis. Tissue ferments are known that hydrolyze the nuclein to the component purin bases, the pyrimidin derivatives (thymin, uracile, cytosin), pentose, and the phosphoric acid. Our knowledge of the subsequent fermentative relations is confined to the purin bases. These are apparently adenine and guinine, the two amido-purin bases. In the acid hydrolysis of nuclein, the purin bases yielded are adenine and guanine alone. These are converted into hypoxanthin and xanthin by the disamidation ferment first described by Jones. There is disagreement between the different investigators whether the ferments that convert the adenine and the guanine are identical; the point has no importance for the theory of the catabolism of nuclein. The reactions for this disamidation are as follows:



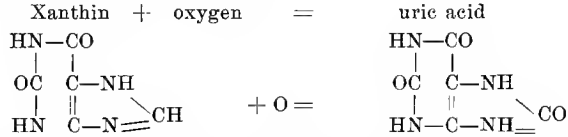
Tissues contain a ferment that accelerated the oxidation of the hypoxanthin, as follows:

Hypoxanthin + oxygen = xanthin.



Tissues contain a ferment that accelerated the oxidation of xanthin to uric acid, as follows:

Xanthin + oxygen = uric acid



Following this, we recognize further a uricolytic ferment in tissues that are active in the cleavage of uric acid; the relations are not understood, but the products seem to be urea and glycocoll. This is in harmony with the older observations, that uric acid is converted into urea in the body.

Recent investigations tend to indicate that when uric acid is oxidized in tissues glyoxylic acid is formed as an intermediary product. Since the final product is urea, the reaction may be formulated somewhat as follows: (*a*) Uric acid + water + oxygen = glyoxyl-urea + ammonia + carbon dioxide; following which the glyoxyl-urea would be oxidized and the ammonia groups united with the carbonyl groups to form two molecules of urea. (*b*) Uric acid + water + oxygen = allantoin (glyoxyl-diureid), which would on hydrolysis yield urea and allanturic acid, which in turn would be hydrolyzed to urea and glyoxylic acid. The experiments succeed with freshly excised or perfused tissues.

The known facts indicating that the catabolism of nuclein is a series of successive fermentative reactions may be summarized as follows:

Nucleo-albumin + water = protein + nuclein.

A proteolytic ferment.

Nuclein + water = pentose, pyrimidins, amido-purins, phosphoric acid.

The ferment has been termed a nuclease.

Amido purins + water = purin bases + ammonia.

The desamidation ferment.

Purin bases + oxygen = uric acid.

An oxydase.

Uric acid + oxygen = urea.

Uricolytic ferment.

The foregoing considerations apply only to the nucleinic catabolism. Whether this is the sole endogenous source of the purin bases of the urine is not known. It is chemically possible that there may be a synthetic formation of purin bases, independent of the oxidation of the bases derived from the hydrolysis of the nuclein; it is, however, undemonstrated. According to Burian, the active muscle forms purin bases (and also creatinine), and this cannot be derived from the nuclein.

THE CARBOHYDRATE METABOLISM.

The polysaccharides of the diet are accelerated in their hydrolysis to maltose by the amylase of the saliva, the pancreatic juice, and the succus entericus. The maltose is fermented by the maltase of the saliva, the pancreatic juice, and the succus entericus. The cane sugar is fermented by the invertase of the saliva, the pancreatic juice, and the succus entericus; the milk sugar is fermented by the lactase of the succus entericus. The products—d-glucose, d-laevulose, and d-galactose—are absorbed and recondensed into glycogen. This reconstruction of the polysaccharide from the hexoses we may assume to be the result of the acceleration of the reversed reaction by a tissue ferment, a glycogenase. Whether this formation of glycogen occurs in the mucosa of the intestine as well as in the liver is not known. There is some evidence that it occurs also in the muscles. Between d-glucose and glycogen a relation of equilibrium seems to exist, again an expression of the presence of a ferment. Whenever the glycogen becomes excessive, fat is formed. The fat is probably formed from the sugar, not from glycogen. The reaction by which fat is formed from sugar is not known. In many plants the reaction is

fermentative and reversible. It is possible that a disturbance of the d-glucose-glycogen equilibrium occurs in diabetes.

When glycogen is hydrolyzed, only d-glucose is obtained. Since d-glucose, d-fructose, and d-galactose are all known to be formers of glycogen, it is probable that before the hexose is condensed to the polysaccharide, the d-fructose and the d-galactose are converted into d-glucose. This polymerization may be easily accomplished as a catalytic reaction by the action of hydroxyl ions, and apparently this polymerization is to be observed in the common hydrolysis of starch. Whether this conversion of the d-fructose and the d-galactose into d-glucose occurs in the intestinal wall or in the liver is not known. The body possesses the power of reversing this reaction. D-galactose is an essential sugar of the body, being present not only in the milk, but also in the lipoids of the central nervous system. In both of these situations it is formed from the d-glucose of the blood.

When saccharose, lactose, and maltose are injected into the circulation they are eliminated in the urine quantitatively, and this indicates that the blood contains no invertase, lactase, or maltase. When glycogen is formed from d-glucose, we have the formation of a polysaccharide. Between the polysaccharide and the hexose there is for all the known vegetable starches a disaccharide stage; whether this exists in the case of glycogen is not demonstrated.

The mechanism of the combustion of sugar is not known. According to Cohnheim, the combustion is the result of the action of two substances: one contained in the muscle, the other in the pancreas. The muscle substance is the accelerating agent; the pancreatic substance simply activates the substance in the muscle. Cohnheim's statement has been contradicted, and we must await confirmation. In the event of confirmation, the relation of the pancreatic substance would be either that of an activator, like enterokinase, or that of an zymo-exciter. The chemical reaction of the combustion of sugar is unknown. Stoklasa has described in muscle and other tissues an alcoholic ferment, and suggests that in the combustions of sugar we have superimposed an alcoholic and an oxidation fermentation, the sugar passing through the stages of lactic acid and alcohol to carbon dioxide and water.

In support of this hypothesis is the fact that traces of lactic acid and alcohol are to be found in all fresh tissues. Muscle, as Hermann demonstrated, will produce carbon dioxide in an atmosphere free of oxygen. The experimental findings that in the autolysis of muscle the alcohol is not increased, though sugar is destroyed and carbon dioxide produced, does not speak against the Stoklasa scheme, since the alcohol, being but an intermediary stage, would be expected to be present only in momentary traces. What makes one pause in the acceptance of the hypothesis are the magnitudes concerned. If the entire combustion of sugar be supposed to pass through the stage of alcohol, that means that for the average body each kilo of active tissue (excluding the fat and skeleton) would need to burn 0.010 g. of alcohol per minute. Of course in such a reaction the alcohol would exhibit only a momentary transitory appearance; nevertheless the quantities concerned are such as will make one hesitate. The hypothesis requires confirmation, but if it can be confirmed, a long step in advance will have been made. Whatever the reaction of combustion, there can be no question that it is a fermentative accelerative.

On the assumption that the combustion of sugar passes through the stage of aethyl alcohol, Stoklasa and Bach suggest the following scheme of progression:

glucose = lactic acid.
$CH_2.OH(CHOH)_4COH = 2\ CH_3.CH(OH).COOH.$
lactic acid = aethyl alcohol + carbon dioxide.
$2\ CH_3.CH(OH).COOH = 2\ CH_3.CH_2.OH + CO_2.$
aethyl alcohol + oxygen = acetic acid + water.
$2\ CH_3.CH_2.OH + O_2 = 2\ CH_3.COOH + 2\ H_2O.$
acetic acid = methane + carbon dioxide.
$2\ CH_3.COOH = 2\ CH_4 + 2\ CO_2.$
Methane + oxygen = formic acid + water.
$2\ CH_4 + 3\ O_2 = 2\ H.COOH + 2\ H_2O.$
formic acid + oxygen = carbon dioxide + water.
$2\ H.COOH + 2\ O_2 = 2\ CO_2 + 2\ H_2O.$

This scheme is to some extent possibly corroborated by the fact that in the early stages of an alcoholic fermentation the production of alcohol is relatively greater than that of carbon dioxide. Should this scheme be confirmed it will afford an opportunity to

determine at just what point if any below the stage of sugar the combustion in the diabetic is impaired.

THE FAT METABOLISM.

The digestion of fat is the simplest reaction of hydrolysis. The absorption of the products, however, is less well understood. Physiologists have for years been resolved into two camps with regard to the digestion and absorption of fats. Bernard first observed the emulsification produced by the action of the pancreatic juice, and this state of emulsification has been long regarded as an important fact in the theory of fat absorption. This emulsion is due but in part to the presence and action of the pancreatic juice; it depends in part upon the soaps and upon the bile. On the other hand, evidence has been accumulating tending to show that the hydrolysis of the fat is a necessary condition preparatory to absorption. The direct proof of the synthesis of fat is afforded by the experiment of injecting free fatty acid and glycerine; they are absorbed as fat, and this combination may be effected by the resected intestinal mucosa. Within recent years the facts have become so clear that we are now warranted in reaching a conclusion. This conclusion, to which the work of Pflueger in late years has contributed much, is that all fats are hydrolyzed before absorption, and that they are not absorbed at all as finely divided particles in the state of emulsification. The microscopic pictures that were formerly relied upon by the Haidenhain school to demonstrate the direct absorption of fat particles are seen on careful investigation to be easily capable of different interpretation. Furthermore, many instances have been adduced in which finely divided bodies in a state of emulsification, as paraffine, have been introduced into the intestine, but never absorbed. On the other hand, the work of Rosenfeld and others has shown that when a peculiar fat is introduced into the intestine and absorbed, that particular fat is to be found chemically intact in the flesh of the animal. Thus linseed oil and erucaic oil are to be found in the liver following their ingestion, and it is possible to feed a starved dog with mutton solely and have firm mutton fat deposited all over the dog, conferring upon

the animal a hardness to the touch very different from the normal condition. The total evidence upon fat metabolism and deposition leads to the postulation of two rules that may be accepted as well established. When animals construct their fats from their natural diet, in the case of herbivora from carbohydrates, the fat is specific to the species. Thus horses and cattle on the same diet of grasses and grain will construct different fats. When, on the other hand, animals ingest fats, that fat is deposited in the tissues unaltered. Thus the fat of a dog may be made to consist solely of mutton fat. In a word, synthesized fat is specific to the species, absorbed fat to the diet. Since this is true, it is apparent that upon the view that fats are absorbed not directly but only after cleavage, we must assume that after the fats are hydrolyzed, in the act of absorption (after passing the [for fats] nonpermeable membrane), they are again recombined into their original states. This is the only interpretation that will correspond to the facts, and do justice to the theory that fats are only absorbed after cleavage. Now we know of but one way in which this combination of the fatty acid and glycerine may be accomplished, and that is by a reversion of the reaction accelerated by the fat-splitting ferment. With the probability of this proposition we are entirely satisfied, since instances of fat synthesis through the reversed action of lipase have been demonstrated. But the details are not at all clear. This combination of the fatty acid and glycerine must be conceded to be effected with great rapidity, since large quantities of fat may be absorbed within a few hours and the contents of the thoracic duct contain no free fatty acid. It is therefore necessary to postulate the presence of some condition very favorable to the reversed action of the ferment, such as a zymo-exciter. We are, however, lacking in information of a chemical nature bearing upon such a condition. There is also the further difficulty that we are not able to understand the rapidity with which the fatty acids must pass the mucous membrane. The higher fatty acids diffuse very slowly, yet in actual digestions large quantities pass through the intestinal wall within a few hours. Naturally one considers the possibility of combinations of the fatty acids with other substances, combinations possessing a greater rate of diffusion. But what sub-

stances? Not soaps, for they diffuse practically not at all, as Krafft has shown. Pflueger has indicated combinations with some of the constituents of the bile, combinations subject to a high degree of hydrolytic dissociation. But such combinations have not been isolated and shown to possess rapid rates of diffusion. Pflueger laid great stress upon the conditions that work for the free solubility of the fatty acids and a rapid cleavage, but that does not entirely elucidate the problem of absorption, since this is an act of diffusion, and solubility and diffusibility do not necessarily go hand in hand. Granted therefore that fats are absorbed only after hydrolysis, and that on absorption they are recombined through the agency of the reversed activity of lipase, the rapidity of absorption of the fatty acids and to some extent the rapidity of the fat synthesis are not understood. One cannot forbear the suggestion that possibly the lipoidal contents of the cells of the intestine, the lecethins, etc., may play a prominent rôle in these transactions, but the suggestion is a purely hypothetical one. Nevertheless it seems that the relations of the Nernst hypothesis previously alluded to find direct application to the intestinal mucosa.

Not only do we not understand the rapidity of the absorption of the hydrolyzed fats through the intestinal, we do not fully understand the velocity of intestinal hydrolysis. One of the most striking facts in fat fermentations is the slowness of cleavage with lipases in vitro as compared with the velocity of the same reaction in vivo. This is not true of lipase alone; it seems to hold good for all the animal ferments, and for many of those of vegetable origin. It has thus been long obvious to the students of fermentation that some condition must be present in the body that is not duplicated in the experiment. For pancreatic lipase we have recently been instructed as to the nature of one favorable condition through the observation of Hewlitt that lecethin acts as a powerful zymo-exciter for the ferment. The accelerating action of bile upon the lypolytic function of the pancreatic secretion has been long known, but never before elucidated. Hewlitt has determined that a trace of lecethin will accelerate notably the cleavage of fats by the pancreatic secretion. This influence he has shown cannot be due to the conversion of a

zymogen into active ferment, nor to any alteration in the reaction; nor is it an additive function, since the lecethin alone is inactive. Probably some component of lecethin operates as well as the lecethin, since the action persists after the heating of the lecethin to 199°, a temperature at which this substance is rapidly disintegrated. Bile contains an appreciable quantity of lecethin, and until the contrary is demonstrated, we are justified in ascribing the accelerating action of the bile on pancreatic lypolysis to the zymo-exciting action of lecethin. As illustrating the specific relations that are encountered in these matters, I may say that lecethin has no influence upon the cleavage of fat by the ferment of the castor bean. A somewhat analogous observation for the lipase of the liver is that reported by Magnus. When the solution of lipase was dyalized it lost its activity, though this was at once restored when a little boiled extract of liver was added; *i.e.,* a necessary coöperating substance was removed by the dyalysis. The substance could be roughly isolated; it was precipitable by uranium acetate in the presence of protein, also by basic lead acetate, insoluble in alcohol and aether, and was destroyed by ashing. Obviously the extract of the liver contains a lipase and a zymo-exciter, or, as Magnus calls it, following the nomenclature of Bertrand, a co-ferment.

The conception of a fat hydrolysis as stated for the insoluble higher fats may then be applied to the digestion of fat within the intestine, with, however, several modifications. In the first place, alkali is present to some extent, and during some time (though both have been largely overrated), and thus some of the fatty acids (the products) are combined and for the purposes of the relations in the system removed. This would tend to reduce the concentration of products, and to that extent would facilitate the rate of transformation. Secondly, fatty acid is being constantly removed by resorption, and this would further reduce the concentration of fatty acid in the system. We observe therefore that even under the interpretation of the velocity of a fat-hydrolysis being simply a velocity of diffusion, the removal of the fatty acid (and the glycerine also, it being resorbed) would tend to accelerate the progress of the transformation. It is at the same time clear that were we to consider that the reac-

tions in the zone of contact of the two phases were not practically instantaneous, but to some extent dependent upon the active mass of the substrate and the products, the removal of the products would accelerate greatly the velocity of the transformation. There is without doubt some practical bearing in these considerations. In the problems of every disturbance in fat digestion, particularly in infants and children, one has to consider the absorption from the intestine as well as the hydrolysis on the part of the lipase of the pancreatic juice. Now there are instances where, with obviously faulty absorption of the fats, there is apparently some reduction in the total hydrolysis of the fats, although there may be good evidence that the pancreatic secretions are performing normally their proteolytic and amylytic functions. For these cases the nearest explanation lies directly in line with the facts just alluded to; the lagging in the fat cleavage may be looked upon as the result of the non-removal of the fatty acids by absorption, the acceleration resulting from the removal of the products is wanting, and we have in the child's intestine simply the velocity of the system in a closed chamber. It is therefore very probable that even with an intact functionation of the pancreas, the rate of cleavage of the fats of a normal diet might be so reduced as to become clinically appreciable, simply because of defective resorption of the fatty acids. The disturbances in fat digestion in children are probably in the large majority of cases dependent upon faulty absorption, due to atrophic enteritis, amyloid deposition in the intestine, tuberculosis of mucosa and retroperioneal lymph glands, tuberculous peritonitis, lymphatic hyperplasis of the retroperitoneal and mysenteric glands of unknown origin—and not to any disturbances in the pancreatic functions. In such cases a certain reduction in the cleavage is to be expected, and the most likely explanation is the one just stated.

Of the combustion of fats we possess still less definite information. Whether the fats are burned directly or first converted into sugars is an old question that has not been decided. In the event of a direct utilization of the fat, its combustion would comprise the two reactions of hydrolysis and oxidation. We are not in a position to speculate upon the mechanism of the reaction.

The fatty acids of the series $C_nH_{2n}O_2$ may be regarded as products representing the successive additions of the group CH_2 to the general construction H.COOH, which is the formula for the lowest member of the group, formic acid. Now the direct oxidation of formic acid may be easily shown to follow the reaction: $2\ H.COOH + O = COOH.COOH + H_2O.COOH.COOH + O = H_2O + 2\ CO_2$, oxalic acid being the intermediary product. There is no theoretical reason why the oxidation of the higher fatty acids should not follow a similar course, the groups CH_2 being successively split off under oxidation to water and carbon dioxide. We know by experience that acetic, formic and oxalic acids are formed in oxidations of the higher fatty acids. We have, however, no direct knowledge that these or similar reactions occur in the body; it would not be difficult to represent the formation of the final products by other equations. Thus the fatty acid might be oxidized directly, through the oxyacid; and an intramolecular cleavage would be equally feasible from the chemical point of view. The possibility that the fats are first converted into sugars has long been assumed, though despite numerous attempts it has not been experimentally demonstrated in a conclusive manner. There can be no doubt of the existence of the opposite reaction, the conversion of sugar into fat. Though the steps of this conversion are not known, beyond the fact that fatty acids are very commonly formed in the disintegrations of sugars, the daily experience of the fat-forming power of sugar demonstrates the occurrence of the process. And it is quite natural therefore to assume that the contrary reaction, the formation of sugar from fats, may be a common physiological occurrence, and this assumption is supported by the known occurrence of this reversible reaction in the vegetable organism. Gautier has described these reactions as processes of fermentation, and has suggested equations. Now while it is apparent that the conversion of sugar into fat and the reconversion of fat into sugar may be acts of fermentation and from the point of view of general physiology probably are to be classed as acts of fermentation, we possess absolutely no chemical data tending to the concrete experimental demonstration of the thesis. The combustion of these bodies for the maintenance of the body heat must, however, be

believed to be of fermentative nature; the wide variations in velocity, without variations in concentration and temperature, alone compel us to the designation of fermentation.

The fats of the body are concerned in very important syntheses, the transformations into the lipoidal bodies, including the various lecethins and the complex fatty substances of the central nervous system. These transformations are in general additions of fatty moieties to other complex molecules; of the nature of the reactions we know nothing. So far as we know, these syntheses are very slow processes, solely connected with growth, reproduction of cells and regeneration, in all probability syntheses of slow velocity and not subject to fluctuations. The lipoidal bodies are of the greatest physiological importance, and doubtless constitute bodies of the highest dignity. The little we know of them does not indicate that their formation represent fermentations.

Fats circulate in a soluble and dialyzable state, apparently in the form of some complex combination. This fact, as stated by Connstein, is easy of confirmation. The facts suggest that this soluble and dialyzable state represents the active state of fat in the mass reaction sense, while neutral fat is but the storage state.

Facts are also present in the physiology of the secretion of the digestive juices that suggest strongly fermentative accelerations. As Bayliss and Starling have shown, the secretion of the pancreatic juice is stimulated by the action of a substance formed in the intestinal mucosa, which they term secretin, and which acts by a direct influence upon the cells of the pancreas, to which it is carried by the circulation. Histological studies had previously suggested that the pancreatic cells form and store in their protoplasm granules from which the zymogen is formed, termed therefore prozymogen. Apparently the secretin accelerates the conversion of the prozymogen into zymogen, which is then secreted. Whatever may be the actual relations, the process suggests strongly a fermentative acceleration. The activation of the zymogens into the active ferments likewise appears to be of enzymic nature. Bayliss and Starling have demonstrated for the activation of trypszymogen by enterokinase that the reaction is

one of hydrolysis, that it follows the law of mass action, and that the velocity of activation is proportional to the mass of the enterokinase. Similar relations may be assumed to hold for the processes of activation of the other zymogens.

Another interesting reaction in the secretion of the alimentary juices is to be found in the secretion of hydrochloric acid. Just what the mechanism of this reaction is we do not know. But since the concentration of the available components of the several possible equations for the formation of hydrochloric acid remains constant, and the temperature is constant, the exaggerated formation of hydrochloric acid during digestion must represent a simple act of positive catalysis. Of experimental evidence we have none. But how else is one to interpret the enormous variations in the velocities of these transformations and secretions, if they be not deemed fermentative?

In a discussion of the relations of ferment action to the processes of disease, we possess as yet more uncultivated than cultivated territory. In few lines are the relations sufficiently simple and the data uncontradicted to enable us to define in even general terms the scope that may be properly allotted to fermentations.

In a great many diseases we have coagulation necroses occupying a most prominent position among the lesions. Thus early in diphtheria, pneumonia, tuberculosis and many other infectious conditions we meet with coagulations as essential features of the lesions. The coagulation itself does not seem in any essential manner different from the coagulation of the blood plasma. Now the definition of coagulation as a fermentation rests simply upon the occurrence of the phenomenon in a rapid manner, without dependence upon alterations in concentration of reacting bodies or temperature, and upon the lack of any proportional relation between the quantity of the active agent and the extent of the transformation. That these coagulations occur slowly in the absence of the accelerator we do not know. Milk has been preserved sterile and neutral for years, without any curdling having developed. Thus we judge these phenomena as being fermentative

without the occurrence of the reaction or process in the original system being known or even assumed. Granted, however, that coagulation at low temperature as the result of the addition of some cellular extract is an act of fermentation, these pathological coagulations have the same rank. These coagulations occur with great velocity, and may affect tissues in a widespread fashion. Since many of these coagulations occur in connection with bacterial infections, the assumption is usually made that the coagulating ferment is derived from the bacteria directly, or at least derived from the reaction between the germs and the tissues. We are, however, acquainted with instances in which the action of chemical substances results in coagulation necrosis at the site of application.

Liquefactions also occur in the body under conditions that point strongly to their definitions as fermentations. So far as we can observe, these liquefactions (decoagulations) differ in no known point from the first stage of protein digestion. Some insults to tissues result directly in liquefaction, but it is much more common for it to be preceded by coagulation. The coagulation necroses are usually followed by liquefaction, and it is through this agency that the removal of the coagulated material is effected. Not only does this apply to coagulated protein, it applies to processes of necrobiosis. Whenever cells involved in a pathological lesion undergo processes of disintegration, we have good reasons for the belief that they are first digested and then the soluble products removed. The absorption of pus is a good illustration. Pus does not usually resorb, but this is due rather to the continuation of the activity of the pyogenic agent than to the resistance of the pus itself to digestion. Now under some circumstances, when the pyogenic agent is weak or dies out, the pus is slowly liquefied and the products gradually resorbed. This occurs sometimes in the peritoneal and pleural cavities, in both localities in some instances involving large masses of pus. Some purulent pleural effusions, those due to the pneumococcus in particular, show a very slight virulence, and will undergo complete resorption, even though the quantity of pus be large. The pus cells certainly do wander back into the circulation of their own account. It were conceivable that leucocytes could invade the pus

cavity and return to the general circulation bearing an amorphous load, and the occurrence of this cannot be denied. But the study of these purulent exudates yields so many chemical signs of protein hydrolysis that we are driven to the conclusion that the chief process lies in the digestion of the dead cells in situ, and the removal of the soluble products of this digestion by resorption. The exudate in crupous pneumonia is removed in the same manner. Very little of the consolidated exudate is removed by expectoration; the mass is liquefied and digested and the products removed by resorption. Direct experiments with the pneumonic lung have demonstrated a rapid rate of digestion under these conditions. The same fact has been demonstrated for the uterus in involution and for the degenerated liver. We are, I think, justified in the general assumption that necrobiotic cells are disposed of by means of a veritable digestion. Furthermore, it is known that in the columns of the spinal cord suffering from sclerosis the quantity of myelin and lecethins is greatly reduced, consequences best interpreted as the result of digestion. In the acute fat necrosis of the pancreas we have an instance of a direct exhibition of the action of a ferment. The marked disintegration of the pancreas in many cases of acute pancreatitis may in a similar manner be best explained as the result of the action of the trypsin upon the pancreas itself.

Similar findings are encountered in the degenerations that are produced as the results of poisonings with known chemical substances. In the degenerations of the liver that result from the action of phosphorus and to a less extent arsenic, we find in the presence in the liver and blood of noteworthy quantities of amido acids, direct evidence that digestion processes have been active. Not only this, but the direct experiment with the self-digestion of the phosphorus liver reveals a marked increase in the rate of self-digestion as compared with the normal liver. In acute yellow atrophy and the analogous conditions, we have in all probability complete analogues of these toxic degenerations of the liver.

Tumors sometimes undergo regressive processes with liquefactions that resemble in all respects these digestions of organs. In many instances these processes of reversions in tumors are

undoubtedly the result of bacterial infection, but in some instances the results cannot be so interpreted.

Now it is not a characteristic of all conditions of necrobiosis and coagulation necrosis that the removal of the material is accomplished through the agency of liquefaction and digestion. In particular is this not the case in tuberculosis. The detritus contained in a cold abcess, erroneously termed pus, is extremely resistant to digestion. Though the contents of these cold abcesses are fluid, they incline to persist for years; the amorphous particles and the suspended colloids resist digestion and the persistence of these exudates is often a serious condition. The same thing is true of the caseous material in pulmonary tuberculosis; it may be encysted, encapsulated, and even encalcified, but the material exhibits the greatest integrity. On chemical analysis, the material exhibits an analogous behavior. It is extremely resistant to solution in all media, and extremely resistant to digestions with known ferments. In short, the caseous material conducts itself in the test tube just as it does in the body. That it is a protein is known largely through the content of nitrogen, and through the results of putrefaction and of acid hydrolysis, to which the material is, however, extremely resistant.

We have in the general occurrence of ferments in tissues the best reason for invoking them in the explanation of these phenomena. It is now an experimentally demonstrated fact that autohydrolysis occurs whenever fats, carbohydrates, and proteins are kept in pure water under aseptic conditions. These have been long known for the starches, the disaccharides and the esters, including the fats. I have during the past two years demonstrated this autohydrolysis for casein, globuline, nuecleo-albumine, protamine, and gelatine. These materials behave in this manner not only in the isolated state but also when they are integral parts of tissues. All fresh, undiseased, sterile organs will on standing exhibit auto-digestion, and the autolyses affect not only the protein but likewise the nuclein, the fat, and the carbohydrates. The velocity of these transformations is very slow; it is only under conditions of infection, inflammation, and necrobiosis that the velocity of these transformations is increased. Whether such accelerations be due to the formation of greater quantities of fer-

ment or also to some alteration in attendant conditions (in mass relations) we do not know. It is also possible or probable that among the products of bacterial metabolism are bodies, different from their specific poisons, that act as accelerators.

While the general effects of bacteria upon tissues—the common infective lesions of the cells—are in all probability not specific to the microörganism, since they occur in all infections to some degree and may reasonably be assumed to be of the nature of fermentations; the specific intoxications are not to be so explained. The toxines of bacteria are specific to the microörganism, and not to the host. They are in all probability produced to some extent in whatever medium of life the germ is stationed, in the body of a susceptible animal or upon an artificial culture medium. The formation of the toxine within the microörganism may in all theoretic possibility represent an act of fermentation. Its action upon the host cannot be so explained. This we must simply class with the specific actions of poisons like atropine, hydrocyanic acids, carbon disulphide, ricine, and mercury, as a reaction de novo. There is, however, one general distinction between the bacterial toxines and the usual vegetable and mineral poisons; under favorable conditions the host will develop anti-toxines, whereas anti-bodies to the other poisons are not developed. To this statement there are exceptions. Some bacteria, under the present conditions of experimentation at least, do not provoke the formation of anti-toxines, while a few vegetable poisons, like ricine, do provoke the formation of anti-bodies. The fact that all the known substances that provoke anti-bodies are colloids is in itself of little direct importance. Of the formation of these anti-toxines we have absolutely no chemical knowledge or explanation. There is a vast amount of data bearing upon the physical and chemical relations of toxines to anti-toxines, into which order has been introduced through the quantitative measurements and calculations of Arrhenius and Madsen. Upon the question of the modus operandi of the formation of the anti-toxine after the injection of the toxine we possess no chemical or physical data. The hypotheses of Ehrlich have thus far not been productive of chemical or physical results.

[ERRATUM.—On page 300, fifth line from bottom of page, instead of CH_2 group read CH — group.]

ANTHROPOLOGICAL MEMOIRS. (Quarto).

Vol. I. Explorations in Peru, by Max Uhle (in preparation).
No. 1. The Ruins of Moche.
No. 2. Huamachuco, Chincha, Ica.
No. 3. The Inca Buildings of the Valley of Pisco.

ASTRONOMY.—W. W. Campbell, Editor. (Lick Observatory, Mt. Hamilton, Cal.)

Publications of the Lick Observatory.—Volumes I-V completed. Volume VI (in progress).

No. 1. A Short Method of Determining Orbits from Three Observations, by A. O. Leuschner.

No. 2. Elements of Asteroid 1900 GA, by A. O. Leuschner and Adelaide M. Hobe.

No. 3. Preliminary Elements of Comet 1900 III, by R. H. Curtiss and C. G. Dall.

Contributions from the Lick Observatory.—Nos. I-V.

Lick Observatory Bulletins.—Volumes I-III completed. Volume IV (in progress).

BOTANY.—W. A. Setchell, Editor. Price per volume $3.50. Volume I (pp. 418) completed. Volume II (in progress).

No. 8. The Resistance of Certain Marine Algæ to Changes in Osmotic Pressure and Temperature, by W. J. V. Osterhout.

No. 9. The Rôle of Osmotic Pressure in Marine Plants, by W. J. V. Osterhout.

No. 10. On the Importance of Physiologically Balanced Solutions for Plants, by W. J. V. Osterhout.

No. 11. The Antitoxic Action of Potassium on Magnesium, by W. J. V. Osterhout.

In one cover. Price .25

No. 12. Cytological Studies in Cyanophyceæ, by Nathaniel Lyon Gardner. Price, 1.00

No. 13. On a small Collection of Mosses from Alaska, by J. Cardot and T. Thériot. Price, .10

EDUCATION.—Edited by the Department of Education. Price per volume $2.50.

Volume I (pp. 424). Notes on the Development of a Child, by Milicent W. Shinn. Price, 2.25

Vol. II (in progress).—No. 1. Notes on Children's Drawings, by Elmer E. Brown. Price, .50

Vol. III (in progress).—No. 1. Origin of American State Universities, by Elmer E. Brown. Price, .50

No. 2. State Aid to Secondary Schools, by David Rhys Jones. Price, .75

ENGINEERING.—Edited under the direction of the Engineering Departments. This series will contain contributions from the Colleges of Mechanics, Mining, and Civil Engineering. Volume I in progress.

No. 1. Bulletin of the Department of Civil Engineering. I.—Moment Diagrams and Typical Live Loads, by Charles Derleth, Jr., Pages 1-9, Plate 1. January, 1907. Price, .10

GEOLOGY.—Bulletin of the Department of Geology. Andrew C. Lawson, Editor. Price per volume $3.50. Volumes I (pp. 428), II (pp. 450), III (475) and IV (462), completed. Volume V (in progress).

1. Carnivora from the Tertiary Formations of the John Day Region, by John C. Merriam. Price, .60

2. Some Edentate-like Remains from the Mascall Beds of Oregon, by William J. Sinclair.

3. Fossil Mollusca from the John Day and Mascall Beds of Oregon, by Robert E. C. Sterns.

In one cover. Price .15

4. New Cestracionf Teeth from the West American Triassic, by Edna M. Wemple. Price, .10

5. Preliminary Note on a New Marine Reptile from the Middle Triassic of Nevada, by John C. Merriam. Price, .10

CLASSICAL PHILOLOGY.—Edward B. Clapp, William A. Merrill, Herbert C. Nutting, Editors. Price per volume $2.00. Volume I (in progress).

No. 1. Hiatus in Greek Melic Poetry, by Edward B. Clapp. Price, $0.50
No. 2. Studies in the Si-clause, by Herbert C. Nutting. " .60
No. 3. The Whence and Whither of the Modern Science of Language, by Benj. Ide Wheeler " .25
No. 4. On the Influence of Lucretius on Horace, by William A. Merrill " .25
No. 5. The Priests of Asklepios (A New Method of Dating Athenian Archons), by William Scott Ferguson " .50

PHILOSOPHY.—Volume I, completed. Price, $2.00.

PHYSIOLOGY.—Jacques Loeb, Editor. Price per volume $2.00. Volume I (pp. 217) completed. Volume II (pp. 215) completed. Volume III (in progress)

No. 5. The Toxicity of Atmospheric Oxygen for the Eggs of the Sea-Urchin (*Strongylocentrotus Purpuratus*) After the Process of Membrane Formation, by Jacques Loeb.
No. 6. On the Necessity of the Presence of Free Oxygen in the Hypertonic Sea-Water for the Production of Artificial Parthenogenesis, by Jacques Loeb.
} In one cover.

No. 7. On the Counteraction of the Toxic Effect of Hypertonic Solutions upon the Fertilized and Unfertilized Egg of the Sea-Urchin by Lack of Oxygen, by Jacques Loeb.

John Bruce MacCallum: On the Mechanism of the Physiological Action of the Cathartics. Price, 75c.

ZOOLOGY.—W. E. Ritter, Editor. Price per volume $3.50. Volumes I completed. Volume II completed. Volume III (in progress). Commencing with Volume II, this series contains Contributions from the Laboratory of the Marine Biological Association of San Diego.

No. 5. Additions to the Copepod Fauna of the San Diego Region, by Calvin Olin Esterly. Pages 40, Plates 5. Price, .35
No. 6. Dinoflagellata of the San Diego Region, II. On *Triposolenia*, a New Genus of the Dinophysidae, by Charles Atwood Kofoid. Pages 24, Plates 3.
No. 7. A Discussion of the Species Characters in Triposolenia, by Charles Atwood Kofoid. Pages 10.
No. 8. On the Significance of the Asymmetry in Triposolenia, by Charles Atwood Kofoid. Pages 7.
} In one cover, Price .35

No. 9. Ostracoda of the San Diego Region. II.—Littoral Forms, by Chancey Juday. Pages 22, Pls. 3.
No. 10. Cladocera of the San Diego Region, by Chancey Juday. Pages 2, text-figure 1.
} In one cover. Price .25

UNIVERSITY CHRONICLE.—An official record of University life, issued quarterly, edited by a committee of the faculty. Price, $1.00 per year. Current volume No. IX.

Address all orders, or requests for information concerning the above publications, to **The University Press, Berkeley, California.**

European agent for the series in American Archaeology and Ethnology, Classical Philology, Education, Philosophy, and Semitic Philology, **Otto Harrassowitz, Leipzig.** For the series in Botany, Geology, Pathology, Physiology, and also American Archaeology and Ethnology, **R. Friedländer & Sohn, Berlin.**